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International application number: PCT/GB05/000987

International filing date: 15 March 2005 (15.03.2005)

Document type: Certified copy of priority document

Document details: Country/Office: GB
Number: 0405893.9
Filing date: 16 March 2004 (16.03.2004)

Date of receipt at the International Bureau: 09 May 2005 (09.05.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

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17MAR04 E881451-1 002029
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| 2. Patent application number *(The Patent Office will fill in his part)* | 0405893.9 16 MAR 2004 |
| 3. Full name, address and postcode of the or of each applicant *(underline all surnames)*<br><br>Patents ADP number *(if you know it)*<br><br>If the applicant is a corporate body, give the country/state of its corporation. | Glaxo Group Limited<br>Glaxo Wellcome House, Berkeley Avenue,<br>Greenford, Middlesex UB6 0NN, Great Britain<br><br>07358700 3<br><br>United Kingdom |
| 4. Title of the invention | Compounds |
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**Signature(s)** A C Connell

A C Connell

**Date:** 16-Mar-04

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# COMPOUNDS

The present invention relates to pyrazolo[3,4-b]pyridine compounds, processes for their preparation, intermediates usable in these processes, and pharmaceutical compositions containing the compounds. The invention also relates to the use of the pyrazolo[3,4-b]pyridine compounds in therapy, for example as inhibitors of phosphodiesterase type IV (PDE4) and/or for the treatment and/or prophylaxis of inflammatory and/or allergic diseases such as chronic obstructive pulmonary disease (COPD), asthma, rheumatoid arthritis or allergic rhinitis.

## Background to the Invention

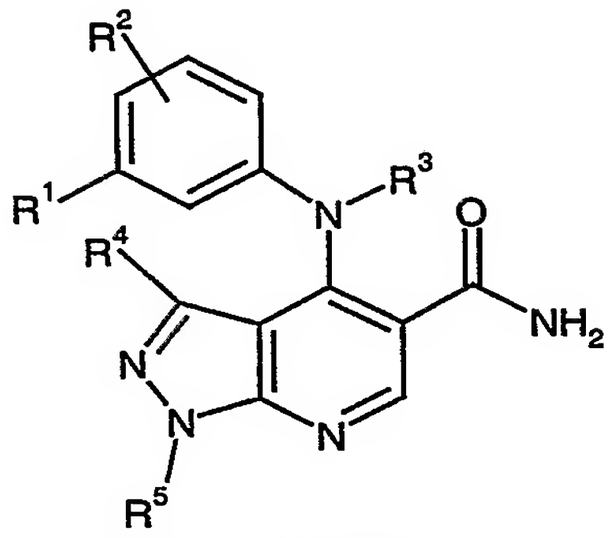
US 3,979,399, US 3,840,546, and US 3,966,746 (E.R.Squibb & Sons) disclose 4-amino derivatives of pyrazolo[3,4-b]pyridine-5-carboxamides wherein the 4-amino group $NR_3R_4$ can be an acyclic amino group wherein $R_3$ and $R_4$ may each be hydrogen, lower alkyl (e.g. butyl), phenyl, etc.; $NR_3R_4$ can alternatively be a 3-6-membered heterocyclic group such as pyrrolidino, piperidino and piperazino. The compounds are disclosed as central nervous system depressants useful as ataractic, analgesic and hypotensive agents.

US 3,925,388, US 3,856,799, US 3,833,594 and US 3,755,340 (E.R.Squibb & Sons) disclose 4-amino derivatives of pyrazolo[3,4-b]pyridine-5-carboxylic acids and esters. The 4-amino group $NR_3R_4$ can be an acyclic amino group wherein $R_3$ and $R_4$ may each be hydrogen, lower alkyl (e.g. butyl), phenyl, etc.; $NR_3R_4$ can alternatively be a 5-6-membered heterocyclic group in which an additional nitrogen is present such as pyrrolidino, piperidino, pyrazolyl, pyrimidinyl, pyridazinyl or piperazinyl. The compounds are mentioned as being central nervous system depressants useful as ataractic agents or tranquilisers, as having antiinflammatory and analgesic properties. The compounds are mentioned as increasing the intracellular concentration of adenosine-3',5'-cyclic monophosphate and for alleviating the symptoms of asthma.

H. Hoehn et al., *J. Heterocycl. Chem.*, 1972, 9(2), 235-253 discloses a series of 1*H*-pyrazolo[3,4-b]pyridine-5-carboxylic acid derivatives with 4-hydroxy, 4-chloro, 4-alkoxy, 4-hydrazino, and 4-amino substituents.

CA 1003419, CH 553 799 and T.Denzel, *Archiv der Pharmazie*, 1974, 307(3), 177-186 disclose 4,5-disubstituted 1*H*-pyrazolo[3,4-b]pyridines unsubstituted at the 1-position.

Japanese laid-open patent application JP-2002-20386-A (Ono Yakuhin Kogyo KK) published on 23 January 2002 discloses pyrazolopyridine compounds of the following formula:



JP-2002-20386-A
(Ono)

wherein $R^1$ denotes 1) a group $-OR^6$, 2) a group $-SR^7$, 3) a C2-8 alkynyl group, 4) a nitro group, 5) a cyano group, 6) a C1-8 alkyl group substituted by a hydroxy group or a C1-8 alkoxy group, 7) a phenyl group, 8) a group $-C(O)R^8$, 9) a group $-SO_2NR^9R^{10}$, 10) a group $-NR^{11}SO_2R^{12}$, 11) a group $-NR^{13}C(O)R^{14}$ or 12) a group $-CH=NR^{15}$. $R^6$ and $R^7$ denote i) a hydrogen atom, ii) a C1-8 alkyl group, iii) a C1-8 alkyl group substituted by a C1-8 alkoxy group, iv) a trihalomethyl group, v) a C3-7 cycloalkyl group, vi) a C1-8 alkyl group substituted by a phenyl group or vii) a 3-15 membered mono-, di- or tricyclic hetero ring containing 1-4 nitrogen atoms, 1-3 oxygen atoms and/or 1-3 sulphur atoms. $R^2$ denotes 1) a hydrogen atom or 2) a C1-8 alkoxy group. $R^3$ denotes 1) a hydrogen atom or 2) a C1-8 alkyl group. $R^4$ denotes 1) a hydrogen atom, 2) a C1-8 alkyl group, 3) a C3-7 cycloalkyl group, 4) a C1-8 alkyl group substituted by a C3-7 cycloalkyl group, 5) a phenyl group which may be substituted by 1-3 halogen atoms or 6) a 3-15 membered mono-, di- or tricyclic hetero ring containing 1-4 nitrogen atoms, 1-3 oxygen atoms and/or 1-3 sulphur atoms. $R^5$ denotes 1) a hydrogen atom, 2) a C1-8 alkyl group, 3) a C3-7 cycloalkyl group, 4) a C1-8 alkyl group substituted by a C3-7 cycloalkyl group or 5) a phenyl group which may be substituted by 1-3 substituents. In group $R^3$, a hydrogen atom is preferred. In group $R^4$ , methyl, ethyl, cyclopropyl, cyclobutyl or cyclopentyl are preferred. The compounds of JP-2002-20386-A are stated as having PDE4 inhibitory activity and as being useful in the prevention and/or treatment of inflammatory diseases and many other diseases.

1,3-Dimethyl-4-(arylamino)-pyrazolo[3,4-b]pyridines with a 5-$C(O)NH_2$ substituent similar or identical to those in JP-2002-20386-A were disclosed as orally active PDE4 inhibitors by authors from Ono Pharmaceutical Co. in: H. Ochiai et al., *Bioorg. Med. Chem. Lett.*, 5th January 2004 issue, vol. 14(1), pp. 29-32 (available on or before 4th December 2003 from the Web version of the journal: "articles in press").

EP 0 076 035 A1 (ICI Americas) discloses pyrazolo[3,4-b]pyridine derivatives as central nervous system depressants useful as tranquilisers or ataractic agents for the relief of anxiety and tension states.

The compound cartazolate, ethyl 4-(n-butylamino)-1-ethyl-1H-pyrazolo[3,4-b]-pyridine-5-carboxylate, is known. J.W. Daly et al., *Med. Chem. Res.*, 1994, 4, 293-306 and D. Shi et al., *Drug Development Research*, 1997, 42, 41-56 disclose a series of 4-

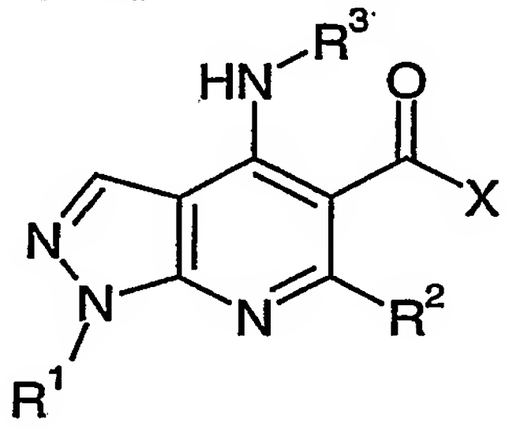
(amino)substituted 1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid derivatives, including ethyl 4-cyclopentylamino-1-methyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate, and their affinities and antagonist activities at $A_1$- and $A_{2A}$-adenosine receptors, and the latter paper discloses their affinities at various binding sites of the $GABA_A$-receptor channel.

S. Schenone et al., *Bioorg. Med. Chem. Lett.*, 2001, 11, 2529-2531, and F. Bondavalli et al., *J. Med. Chem.*, 2002, vol. 45 (Issue 22, 24 October 2002, allegedly published on Web 09/24/2002), pp. 4875-4887 disclose a series of 4-amino-1-(2-chloro-2-phenylethyl)-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid ethyl esters as $A_1$-adenosine receptor ligands.

WO 02/060900 A2 appears to disclose, as MCP-1 antagonists for treatment of allergic, inflammatory or autoimmune disorders or diseases, a series of bicyclic heterocyclic compounds with a $-C(O)-NR^4-C(O)-NR^5R^6$ substituent, including isoxazolo[5,4-b]pyridines and 1*H*-pyrazolo[3,4-b]pyridines (named as pyrazolo[5,4-b]pyridines) with the $-C(O)-NR^4-C(O)-NR^5R^6$ group as the 5-substituent and optionally substituted at the 1-, 3-, 4-, and/or 6-positions. Bicyclic heterocyclic compounds with a $-C(O)NH_2$ substituent instead of the $-C(O)-NR^4-C(O)-NR^5R^6$ substituent are alleged to be disclosed in WO 02/060900 as intermediates in the synthesis of the $-C(O)-NR^4-C(O)-NR^5R^6$ substituted compounds.

WO 00/15222 (Bristol-Myers Squibb) discloses *inter alia* pyrazolo[3,4-b]pyridines having *inter alia* a $C(O)-X_1$ group at the 5-position and a group $E_1$ at the 4-position of the ring system. Amongst other things, $X_1$ can for example be $-OR_9$, $-N(R_9)(R_{10})$ or $-N(R_5)(-A_2-R_2)$, and $E_1$ can for example be $-NH-A_1$-cycloalkyl, $-NH-A_1$-substituted cycloalkyl, or $-NH-A_1$-heterocyclo; wherein $A_1$ is an alkylene or substituted alkylene bridge of 1 to 10 carbons and $A_2$ can for example be a direct bond or an alkylene or substituted alkylene bridge of 1 to 10 carbons. The compounds are disclosed as being useful as inhibitors of cGMP phosphodiesterase, especially PDE type V, and in the treatment of various cGMP-associated conditions such as erectile dysfunction. Compounds with a cycloalkyl or heterocyclo group directly attached to -NH- at the 4-position of the pyrazolo[3,4-b]pyridine ring system and/or having PDE4 inhibitory activity do not appear to be disclosed in WO 00/15222.

Copending unpublished patent application PCT/EP03/11814, filed on 12 September 2003 in the name of Glaxo Group Limited, discloses pyrazolo[3,4-b]pyridine compounds or salts thereof with a $4-NHR^3$ group and a 5-C(O)-X group, according to this formula (I):



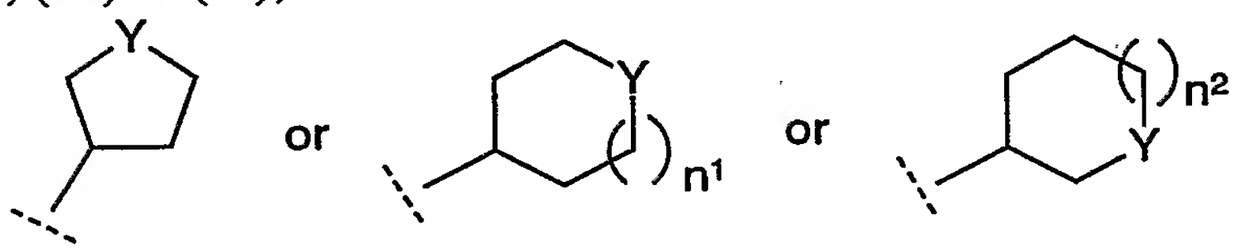
(I)

wherein:

$R^1$ is $C_{1-4}$alkyl, $C_{1-3}$fluoroalkyl, $-CH_2CH_2OH$ or $-CH_2CH_2CO_2C_{1-2}$alkyl;

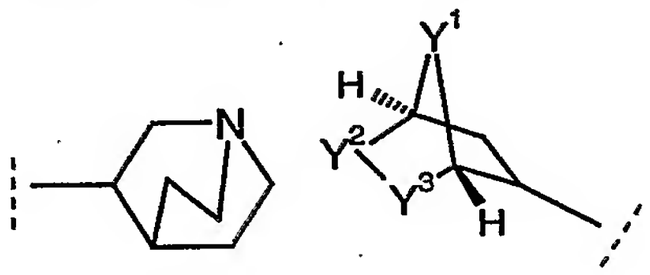
$R^2$ is a hydrogen atom (H), methyl or $C_1$fluoroalkyl;

$R^3$ is optionally substituted $C_{3-8}$cycloalkyl or optionally substituted mono-unsaturated-$C_{5-7}$cycloalkenyl or an optionally substituted heterocyclic group of sub-formula (aa), (bb) or (cc);



(aa) (bb) (cc)

in which $n^1$ and $n^2$ independently are 1 or 2; and in which Y is O, S, $SO_2$, or $NR^{10}$;



(dd) (ee)

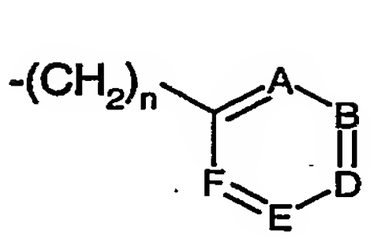
or $R^3$ is a bicyclic group (dd) or (ee): ;

and wherein X is $NR^4R^5$ or $OR^{5a}$.

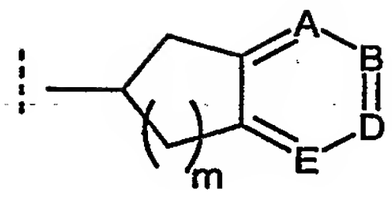
In PCT/EP03/11814, $R^4$ is a hydrogen atom (H); $C_{1-6}$alkyl; $C_{1-3}$fluoroalkyl; or $C_{2-6}$alkyl substituted by one substituent $R^{11}$.

In PCT/EP03/11814, $R^5$ can be: a hydrogen atom (H); $C_{1-8}$alkyl; $C_{1-8}$ fluoroalkyl; $C_{3-8}$cycloalkyl optionally substituted by a $C_{1-2}$alkyl group; $-(CH_2)_n{}^4$-$C_{3-8}$cycloalkyl optionally substituted, in the $-(CH_2)_n{}^4$- moiety or in the $C_{3-8}$cycloalkyl moiety, by a $C_{1-2}$alkyl group, wherein $n^4$ is 1, 2 or 3; $C_{2-6}$alkyl substituted by one or two independent substituents $R^{11}$; $-(CH_2)_n{}^{11}$-$C(O)R^{16}$; $-(CH_2)_n{}^{12}$-$C(O)NR^{12}R^{13}$; $-CHR^{19}$-$C(O)NR^{12}R^{13}$; $-(CH_2)_n{}^{12}$-$C(O)OR^{16}$; $-(CH_2)_n{}^{12}$-C(O)OH; $-CHR^{19}$-$C(O)OR^{16}$; $-CHR^{19}$-C(O)OH; $-(CH_2)_n{}^{12}$-$SO_2$-$NR^{12}R^{13}$; $-(CH_2)_n{}^{12}$-$SO_2R^{16}$; or $-(CH_2)_n{}^{12}$-CN; $-(CH_2)_n{}^{13}$-Het; or optionally substituted phenyl.

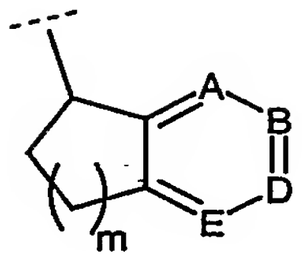
Alternatively, in PCT/EP03/11814, $R^5$ can have the sub-formula (x), (y), (y1) or (z):



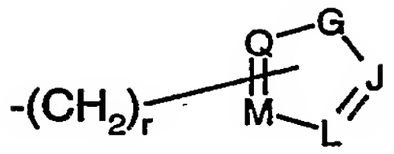
(x)



(y)



(y1)



(z)

wherein in sub-formula (x), n = 0, 1 or 2; in sub-formula (y) and (y1), m = 1 or 2; and in sub-formula (z), r = 0, 1 or 2; and wherein in sub-formula (x) and (y) and (y1), none, one or two of A, B, D, E and F are independently nitrogen or nitrogen-oxide ($N^+$-$O^-$) provided that no more than one of A, B, D, E and F is nitrogen-oxide, and the remaining of A, B, D, E and F are independently CH or $CR^6$; and provided that when n is 0 in sub-formula (x) then one or two of A, B, D, E and F are independently nitrogen or nitrogen-oxide ($N^+$-$O^-$) and no more than one of A, B, D, E and F is nitrogen-oxide;

In PCT/EP03/11814, each $R^6$, independently of any other $R^6$ present, is: a halogen atom; $C_{1-6}$alkyl; $C_{1-4}$fluoroalkyl; $C_{1-4}$alkoxy; $C_{1-2}$fluoroalkoxy; $C_{3-6}$cycloalkyloxy; $-C(O)R^{16a}$; $-C(O)OR^{30}$; $-S(O)_2-R^{16a}$; $R^{16a}-S(O)_2-NR^{15a}-$; $R^7R^8N-S(O)_2-$; $C_{1-2}$alkyl-$C(O)-R^{15a}N-S(O)_2-$; $C_{1-4}$alkyl-$S(O)-$; Ph-$S(O)-$; $R^7R^8N-CO-$; $-NR^{15}-C(O)R^{16}$; $R^7R^8N$; OH; $C_{1-4}$alkoxymethyl; $C_{1-4}$alkoxyethyl; $C_{1-2}$alkyl-$S(O)_2-CH_2-$; $R^7R^8N-S(O)_2-CH_2-$; $C_{1-2}$alkyl-$S(O)_2-NR^{15a}-CH_2-$; $-CH_2-OH$; $-CH_2CH_2-OH$; $-CH_2-NR^7R^8$; $-CH_2-CH_2-NR^7R^8$; $-CH_2-C(O)OR^{30}$; $-CH_2-C(O)-NR^7R^8$; $-CH_2-NR^{15a}-C(O)-C_{1-3}$alkyl; $-(CH_2)_n{}^{14}$-$Het^1$ where $n^{14}$ is 0 or 1; cyano (CN); $Ar^{5b}$; or phenyl, pyridinyl or pyrimidinyl wherein the phenyl, pyridinyl or pyrimidinyl independently are optionally substituted by one or two of fluoro, chloro, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy;

or two adjacent $R^6$ taken together can be $-O-(CMe_2)-O-$ or $-O-(CH_2)_n{}^{14}-O-$ where $n^{14}$ is 1 or 2.

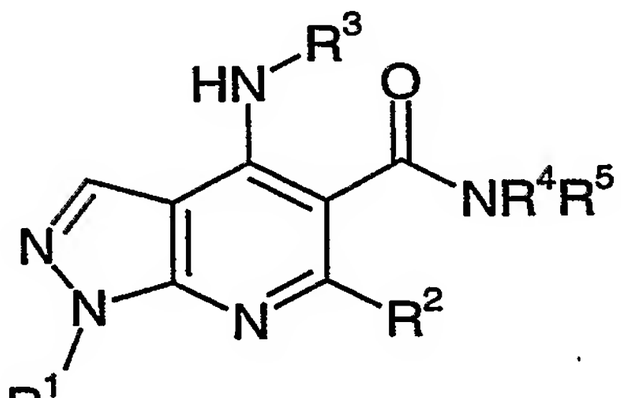
In PCT/EP03/11814, in sub-formula (z), G is O or S or $NR^9$ wherein $R^9$ is a hydrogen atom (H), $C_{1-4}$alkyl or $C_{1-4}$fluoroalkyl; none, one, two or three of J, L, M and Q are nitrogen; and the remaining of J, L, M and Q are independently CH or $CR^6$ where $R^6$, independently of any other $R^6$ present, is as defined therein.

The pyrazolo[3,4-b]pyridine compounds of formula (I) and salts thereof disclosed in PCT/EP03/11814 are disclosed as being inhibitors of phosphodiesterase type IV (PDE4), and as being useful for the treatment and/or prophylaxis of an inflammatory and/or allergic diseases such as chronic obstructive pulmonary disease (COPD), asthma, rheumatoid arthritis, or allergic rhinitis.

**The Invention**

We have now found new pyrazolo[3,4-b]pyridine compounds, which compounds inhibit phosphodiesterase type IV (PDE4).

The present invention therefore provides a compound of formula (I) or a salt thereof (in particular, a pharmaceutically acceptable salt thereof):



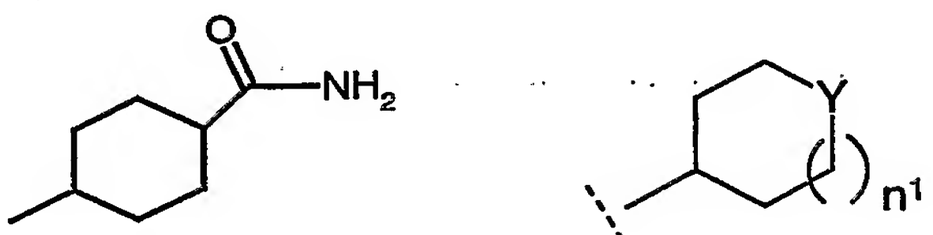
(I)

wherein:

$R^1$ is $C_{1-4}$alkyl, $C_{1-3}$fluoroalkyl, or $-CH_2CH_2OH$;

$R^2$ is a hydrogen atom (H), methyl or $C_1$fluoroalkyl;

$R^3$ is a 4-(aminocarbonyl)cyclohexyl (i.e. 4-(aminocarbonyl)cyclohexan-1-yl) group of sub-formula (aa), or an N-aminocarbonyl-piperidinyl or -pyrrolidinyl group of sub-formula (bb);



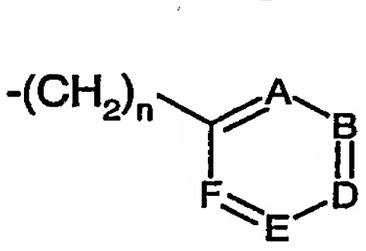
(aa) (bb)

wherein Y is $NHCONH_2$ and $n^1$ is 0 or 1;

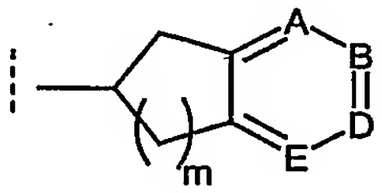
and wherein, the cyclohexyl group of sub-formula (aa) or the piperidinyl or pyrrrolidinyl groups of sub-formula (bb) may be further optionally substituted with one or two substituents independently selected from $C_{1-2}$alkyl; $C_{1-2}$fluoroalkyl; $CH_2OH$; $-C(O)OR^{23}$ wherein $R^{23}$ is H or $C_{1-2}$alkyl; $-C(O)NHR^{23}$; or fluoro; on any ring carbon; as well as, on the C2, C3, C5 and C6 of the cyclohexyl group of (aa), the C2 or C6 of the piperidinyl ring or the C5 of the pyrrolidinyl ring of (bb), a substituent selected from OH; $C_{1-2}$alkoxy; $C_{1-2}$fluoroalkoxy; OH, alkoxy;

$R^4$ is a hydrogen atom (H); $C_{1-6}$alkyl; $C_{1-3}$fluoroalkyl; or $C_{2-6}$alkyl substituted by one substituent $R^{11}$;

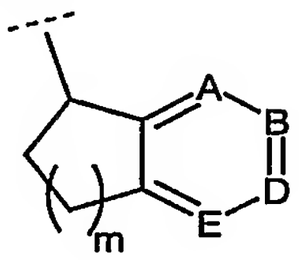
$R^5$ is a group of the sub-formula (x), (y), (y1) or (z):



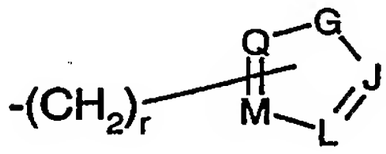
(x)



(y)



(y1)



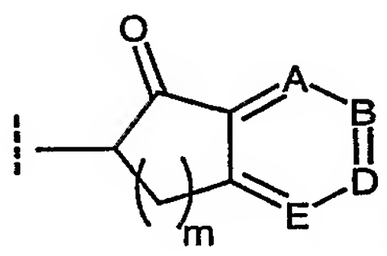
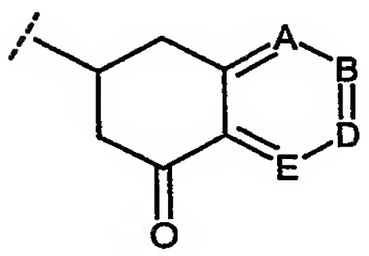
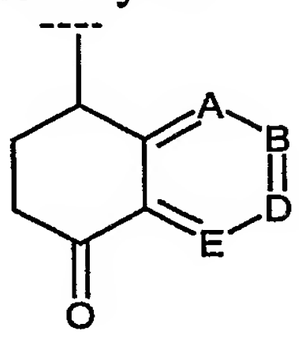
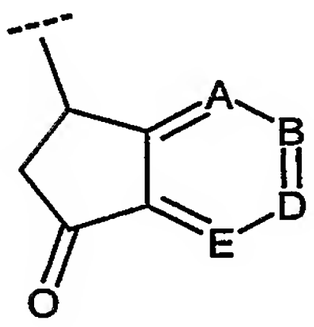
(z)

wherein in sub-formula (x), n = 0, 1 or 2; in sub-formula (y) and (y1), m = 1 or 2; and in sub-formula (z), r = 0, 1 or 2;

wherein in sub-formula (x) and (y) and (y1), none, one or two of A, B, D, E and F are independently nitrogen or nitrogen-oxide ($N^+$-$O^-$) provided that no more than one of A, B, D, E and F is nitrogen-oxide; and the remaining of A, B, D, E and F are independently CH or $CR^6$;

wherein, each $R^6$, independently of any other $R^6$ present, is: a halogen atom; $C_{1-6}$alkyl (e.g. $C_{1-4}$alkyl or $C_{1-2}$alkyl); $C_{1-4}$fluoroalkyl (e.g. $C_{1-2}$fluoroalkyl); $C_{1-4}$alkoxy (e.g. $C_{1-2}$alkoxy); $C_{1-2}$fluoroalkoxy; $C_{3-6}$cycloalkyloxy; -C(O)$R^{16a}$; -C(O)$OR^{30}$; -S(O)$_2$-$R^{16a}$ (e.g. $C_{1-2}$alkylsulphonyl, that is $C_{1-2}$alkyl-$SO_2$-); $R^{16a}$-S(O)$_2$-$NR^{15a}$- (e.g. $C_{1-2}$alkyl-$SO_2$-NH-); $R^7R^8$N-S(O)$_2$-; $C_{1-2}$alkyl-C(O)-$R^{15a}$N-S(O)$_2$-; $C_{1-4}$alkyl-S(O)-, Ph-S(O)-, $R^7R^8$N-CO-; -$NR^{15}$-C(O)$R^{16}$; $R^7R^8$N; OH; $C_{1-4}$alkoxymethyl; $C_{1-4}$alkoxyethyl; $C_{1-2}$alkyl-S(O)$_2$-$CH_2$-; $R^7R^8$N-S(O)$_2$-$CH_2$-; $C_{1-2}$alkyl-S(O)$_2$-$NR^{15a}$-$CH_2$-; -$CH_2$-OH; -$CH_2CH_2$-OH; -$CH_2$-$NR^7R^8$; -$CH_2$-$CH_2$-$NR^7R^8$; -$CH_2$-C(O)$OR^{30}$; -$CH_2$-C(O)-$NR^7R^8$; -$CH_2$-$NR^{15a}$-C(O)-$C_{1-3}$alkyl; -$(CH_2)_n{}^{14}$-$Het^1$ where $n^{14}$ is 0 or 1; cyano (CN); $Ar^{5b}$; or phenyl, pyridinyl or pyrimidinyl wherein the phenyl, pyridinyl or pyrimidinyl independently are optionally substituted by one or two of fluoro, chloro, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy; or where two adjacent $R^6$ taken together are –O–($CMe_2$)–O– or –O–$(CH_2)_n{}^{14}$–O– where $n^{14}$ is 1 or 2;

wherein sub-formula (y) and (y1), independently, are optionally substituted by oxo (=O) at a ring carbon adjacent the 6-membered aromatic ring (for example, sub-formula (y) can

optionally be  or  , or sub-formula (y1) can optionally be  or  );

wherein in sub-formula (z), G is O or S or $NR^9$ wherein $R^9$ is a hydrogen atom (H), $C_{1-4}$alkyl or $C_{1-4}$fluoroalkyl; none, one, two or three of J, L, M and Q are nitrogen; and the remaining of J, L, M and Q are independently CH or $CR^6$ where $R^6$, independently of any other $R^6$ present, is as defined herein;

and wherein:

$R^7$ and $R^8$ are independently a hydrogen atom (H); $C_{1-4}$alkyl (e.g. $C_{1-2}$alkyl such as methyl); $C_{3-6}$cycloalkyl; or phenyl optionally substituted by one or two substituents independently being: fluoro, chloro, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy;

or $R^7$ and $R^8$ together are $-(CH_2)_n{}^6-$ or $-C(O)-(CH_2)_n{}^7-$ or $-C(O)-(CH_2)_n{}^{10}-C(O)-$ or $-(CH_2)_n{}^8-X^7-(CH_2)_n{}^9-$ or $-C(O)-X^7-(CH_2)_n{}^{10}-$ in which: $n^6$ is 3, 4, 5 or 6 (suitably $n^6$ is 4 or 5), $n^7$ is 2, 3, 4, or 5 (suitably $n^7$ is 3 or 4), $n^8$ and $n^9$ and $n^{10}$ independently are 2 or 3 (suitably independently 2), and $X^7$ is O or $NR^{14}$;

$R^{7a}$ is a hydrogen atom (H) or $C_{1-4}$alkyl (suitably H or $C_{1-2}$alkyl, more suitably H or methyl);

$R^{8a}$ is a hydrogen atom (H) or methyl (suitably H);

$R^{14}$, independent of other $R^{14}$, is a hydrogen atom (H); $C_{1-4}$alkyl (e.g. $C_{1-2}$alkyl); $C_{1-2}$fluoroalkyl (e.g. $CF_3$); cyclopropyl; $-C(O)-C_{1-4}$alkyl (e.g. -C(O)Me); $-C(O)NR^{7a}R^{8a}$ (e.g. $-C(O)NH_2$); or $-S(O)_2-C_{1-4}$alkyl (e.g. $-S(O)_2$Me) (preferably, $R^{14}$, $R^{17}$ and/or $R^{17a}$ independently is/are: H; $C_{1-2}$alkyl; or -C(O)Me);

$R^{15}$, independent of other $R^{15}$, is a hydrogen atom (H); $C_{1-4}$alkyl (e.g. $^t$Bu or $C_{1-2}$alkyl e.g. methyl); $C_{3-6}$cycloalkyl; or phenyl optionally substituted by one or two of: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy;

$R^{15a}$, independent of other $R^{15a}$, is a hydrogen atom (H) or $C_{1-4}$alkyl (e.g. H, $^{t}$Bu or $C_{1-2}$alkyl such as methyl; preferably $R^{15a}$ is H or $C_{1-2}$alkyl, more preferably H);

$R^{16a}$ is:
$C_{1-6}$alkyl (e.g. $C_{1-4}$alkyl or $C_{1-2}$alkyl);
$C_{3-6}$cycloalkyl (e.g. $C_{5-6}$cycloalkyl) optionally substituted by one oxo (=O), OH or $C_{1-2}$alkyl substituent (e.g. optionally substituted at the 3- or 4-position of a $C_{5-6}$cycloalkyl ring; and/or preferably unsubstituted $C_{3-6}$cycloalkyl);
$C_{3-6}$cycloalkyl-$CH_2$- (e.g. $C_{5-6}$cycloalkyl-$CH_2$-);
pyridinyl (e.g. pyridin-2-yl) optionally substituted on a ring carbon atom by one of: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy;
$Ar^{5c}$;
phenyl optionally substituted by one or two substituents independently being: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy;
benzyl optionally substituted on its ring by one or two substituents independently being: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy; or
a 4-, 5-, 6- or 7-membered saturated heterocyclic ring connected at a ring-carbon and containing one or two ring-hetero-atoms independently selected from O, S, and N; wherein any ring-nitrogens which are present are present as $NR^{27}$ where $R^{27}$ is H, $C_{1-2}$alkyl or -C(O)Me; and wherein the ring is optionally substituted at carbon by one $C_{1-2}$alkyl or oxo (=O) substituent, provided that any oxo (=O) substituent is substituted at a ring-carbon atom bonded to a ring-nitrogen;

$R^{30}$, independent of other $R^{30}$, is a hydrogen atom (H), $C_{1-4}$alkyl or $C_{3-6}$cycloalkyl;

$Ar^{5b}$ and $Ar^{5c}$ independently is/are a 5-membered aromatic heterocyclic ring containing one O, S or $NR^{15a}$ in the 5-membered ring, wherein the 5-membered ring can optionally additionally contain one or two N atoms, and wherein the heterocyclic ring is optionally substituted on a ring carbon atom by one of: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, -$CH_2$OH, -$CH_2$-O$C_{1-2}$alkyl, OH (including the keto tautomer thereof) or –$CH_2$-$NR^{28}R^{29}$ wherein $R^{28}$ and $R^{29}$ independently are H or methyl; and

$Het^1$ is a 4-, 5-, 6- or 7-membered saturated heterocyclic ring connected at a ring-carbon and containing one or two ring-hetero-atoms independently selected from O, S, and N; wherein any ring-nitrogens which are present are present as $NR^{31}$ where $R^{31}$ is H, $C_{1-2}$alkyl or -C(O)Me; and wherein the ring is optionally substituted at carbon by one $C_{1-2}$alkyl or oxo (=O) substituent, provided that any oxo (=O) substituent is substituted at a ring-carbon atom bonded to a ring-nitrogen.

In compounds, for example in the compounds of formula (I), an "alkyl" group or moiety may be straight-chain or branched. Alkyl groups, for example $C_{1-8}$alkyl or $C_{1-6}$alkyl or $C_{1-4}$alkyl or $C_{1-3}$alkyl or $C_{1-2}$alkyl, which may be employed include $C_{1-6}$alkyl or $C_{1-4}$alkyl or $C_{1-3}$alkyl or $C_{1-2}$alkyl such as methyl, ethyl, n-propyl, n-butyl, n-pentyl, or n-hexyl or any branched isomers thereof such as isopropyl, t-butyl, sec-butyl, isobutyl, 3-methylbutan-2-yl, 2-ethylbutan-1-yl, or the like.

A corresponding meaning is intended for "alkoxy", "alkylene", and like terms derived from alkyl. For example, "alkoxy" such as $C_{1-6}$alkoxy or $C_{1-4}$alkoxy or $C_{1-2}$alkoxy includes methoxy, ethoxy, propyloxy, and oxy derivatives of the alkyls listed above. "Alkylsulfonyl" such as $C_{1-4}$alkylsulfonyl includes methylsulfonyl (methanesulfonyl), ethylsulfonyl, and others derived from the alkyls listed above. "Alkylsulfonyloxy" such as $C_{1-4}$alkylsulfonyloxy includes methanesulfonyloxy (methylsulfonyloxy), ethanesulfonyloxy, *et al.*

"Cycloalkyl", for example $C_{3-8}$cycloalkyl, includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and the like. Preferably, a $C_{3-8}$cycloalkyl group is $C_{3-6}$cycloalkyl or $C_{5-6}$cycloalkyl , that is contains a 3-6 membered or 5-6 membered carbocyclic ring.

"Fluoroalkyl" includes alkyl groups with one, two, three, four, five or more fluorine substituents, for example $C_{1-4}$fluoroalkyl or $C_{1-3}$fluoroalkyl or $C_{1-2}$fluoroalkyl such as monofluoromethyl, difluoromethyl, trifluoromethyl, pentafluoroethyl, 2,2,2-trifluoroethyl ($CF_3CH_2$-), 2,2-difluoroethyl ($CHF_2CH_2$-), 2-fluoroethyl ($CH_2FCH_2$-), etc.
"Fluoroalkoxy" includes $C_{1-4}$fluoroalkoxy or $C_{1-2}$fluoroalkoxy such as trifluoromethoxy, pentafluoroethoxy, monofluoromethoxy, difluoromethoxy, etc.
"Fluoroalkylsulfonyl" such as $C_{1-4}$fluoroalkylsulfonyl includes trifluoromethanesulfonyl, pentafluoroethylsulfonyl, etc.

A halogen atom ("halo") present in compounds, for example in the compounds of formula (I), means a fluorine, chlorine, bromine or iodine atom ("fluoro", "chloro", "bromo" or "iodo"), for example fluoro, chloro or bromo.

When the specification states that atom or moiety A is "bonded" or "attached" to atom or moiety B, it means that atom/moiety A is directly bonded to atom/moiety B usually by means of a covalent bond or a double covalent bond, and excludes A being indirectly attached to B via one or more intermediate atoms/moieties (e.g. excludes A-C-B); unless it is clear from the context that another meaning is intended.

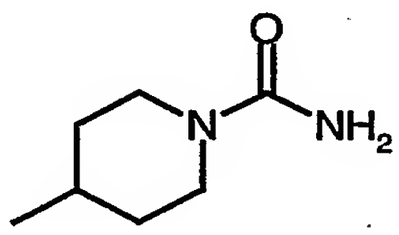
When $R^1$ is $C_{1-4}$alkyl or $C_{1-3}$fluoroalkyl, it can be straight-chained or branched. Where $R^1$ is $C_{1-4}$alkyl then it can for example be methyl, ethyl, n-propyl, isopropyl or n-butyl.

When $R^1$ is $C_{1-3}$fluoroalkyl, then $R^1$ can for example be $C_1$fluoroalkyl such as monofluoromethyl, difluoromethyl, trifluoromethyl; or $R^1$ can be $C_2$fluoroalkyl such as pentafluoroethyl or more preferably $C_1$fluoroalkyl-$CH_2$- such as 2,2,2-trifluoroethyl ($CF_3CH_2$-), 2,2-difluoroethyl ($CHF_2CH_2$-), or 2-fluoroethyl ($CH_2FCH_2$-).

Preferably, $R^1$ is $C_{1-3}$alkyl (e.g. methyl, ethyl or n-propyl), $C_{1-3}$fluoroalkyl or -$CH_2CH_2OH$. $R^1$ is more preferably $C_{1-3}$alkyl, $C_{1-2}$fluoroalkyl, or -$CH_2CH_2OH$. Still more preferably, $R^1$ is $C_{2-3}$alkyl (e.g. ethyl or n-propyl), $C_2$fluoroalkyl (e.g. $C_1$fluoroalkyl-$CH_2$- such as $CF_3$-$CH_2$-) or -$CH_2CH_2OH$; in particular ethyl, n-propyl or -$CH_2CH_2OH$. Yet more preferably, $R^1$ is $C_2$alkyl or $C_2$fluoroalkyl. $R^1$ is most preferably ethyl.

Preferably, $R^2$ is a hydrogen atom (H) or methyl, for example a hydrogen atom (H).

In a preferred embodiment, the group of sub-formula (bb) is:




Preferably, in $R^3$ there is one substituent or no substituent.

In one optional embodiment, in $R^3$, the cyclohexyl group may have no further optional substituents (beyond 4-$CONH_2$). If present, a further optional substituent for the cyclohexyl group in $R^3$, may be present at the 3-, or 5- position.

(In this connection and generally herein, the 1-position of the $R^3$ ring, e.g. of the $R^3$ cyclohexyl ring is deemed to be the connection point to the -NH- in formula (I) = the ring atom connecting to the -NH- in formula (I)).

In one embodiment, in $R^3$, the piperidinyl or pyrrolidinyl group of sub-formula (bb) is not substituted on a ring carbon..

In a further embodiment, in $R^3$, the piperidinyl or pyrrolidinyl group of sub-formula (bb), may be substituted with a substituent selected from OH; $C_{1-2}$alkyl (e.g. methyl) or $C_{1-2}$fluoroalkyl (e.g. $C_1$fluoroalkyl such as -$CH_2F$ or -$CHF_2$).

It will be appreciated that when $R^3$ is substituted, then the substituent can be in the *cis* or *trans* configuration with respect to the -NH- group of formula (I) to which $R^3$ is attached

(bonded). The present invention covers each configuration as well as mixtures of configurations, in particular wherein the stated configuration is the major component. For example, an OH or the $-C(O)NH_2$ substituent on the cyclohexyl can for example be in the *cis* or *trans* configuration with respect to the -NH- group of formula (I) to which $R^3$ is attached (bonded), including mixtures of configurations wherein the stated configuration is the major component.

Preferably, the aminocarbonyl group of sub-formula (aa) is in the *cis* configuration, i.e. preferably it is a *cis*-[4-(aminocarbonyl)cyclohexan-1-yl]amino group.

Preferably, $R^{11}$ is: hydroxy (OH); $C_{1-6}$alkoxy (e.g. $C_{1-4}$alkoxy such as t-butyloxy, ethoxy or methoxy); phenyloxy; benzyloxy; $-NR^{12}R^{13}$; $-NR^{15}-C(O)R^{16}$; $-NR^{15}-C(O)-NH-R^{15}$; or $-NR^{15}-SO_2R^{16}$. More preferably, $R^{11}$ is hydroxy (OH), $C_{1-4}$alkoxy, or $-NR^{12}R^{13}$; still more preferably OH, ethoxy, methoxy, $NH_2$, NHMe, NHEt, or $NMe_2$, yet more preferably OH, methoxy, or $NMe_2$.

Preferably, $R^7$ and/or $R^8$ are independently a hydrogen atom (H); $C_{1-2}$alkyl such as methyl; $C_{3-6}$cycloalkyl; or phenyl optionally substituted by one of: fluoro, chloro, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy; or $R^7$ and $R^8$ together are $-(CH_2)_n{}^6-$ or $-(CH_2)_n{}^8-X^7-(CH_2)_n{}^9-$ wherein $X^7$ is $NR^{14}$ or preferably O.

When $R^7$ is cycloalkyl or optionally substituted phenyl, then preferably $R^8$ is neither cycloalkyl nor optionally substituted phenyl.

Most preferably, $R^7$ and/or $R^8$ independently are a hydrogen atom (H) or $C_{1-2}$alkyl. It is preferable that $R^7$ is a hydrogen atom (H).

Preferably $n^6$ is 4 or 5. Preferably $n^7$ is 2, 3 or 4. Preferably, $n^8$, $n^9$ and/or $n^{10}$ is/are independently 2.

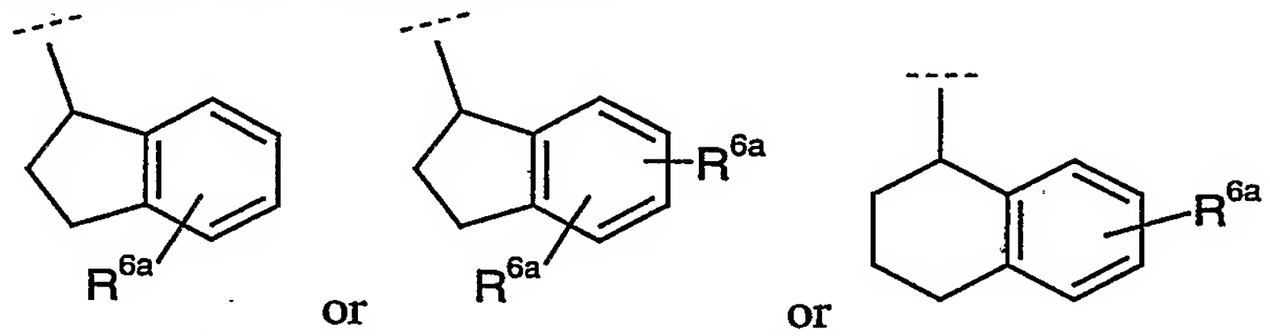
In a preferred embodiment, $R^4$ is hydrogen.

In a preferred embodiment $R^5$ is a group of sub-formula (x) or (z).

In sub-formula (x), it is preferred that none, one or two of A, B, D, E and F are nitrogen; none, one, two or three of A, B, D, E and F are $CR^6$; and the remaining of A, B, D, E and F are CH.. More preferably, none, one or two of A, B, D, E and F are nitrogen; none, one or two of A, B, D, E and F are $CR^6$; and the remaining of A, B, D, E and F are CH.. Yet more preferably, none or one of A, B, D, E and F are nitrogen, and/or preferably none, one or two of A, B, D, E and F are $CR^6$.

Preferably, sub-formula (x) is: benzyl; optionally substituted on the phenyl ring with one or two $R^6$ substituents.

Preferably, sub-formula (y1) is:



wherein $R^{6a}$ is or independently are either $R^6$ as defined herein or preferably hydrogen.

Preferably, in sub-formula (z), none, one or two of J, L, M and Q are nitrogen.

In sub-formula (x), (y) (y1) and/or (z), preferably, each $R^6$, independently of any other $R^6$ present, is a fluorine, chlorine, bromine or iodine atom, methyl, ethyl, n-propyl, isopropyl, $C_4$alkyl, trifluoromethyl, $-CH_2OH$, methoxy, ethoxy, $C_1$fluoroalkoxy (e.g. trifluoromethoxy or difluoromethoxy), OH, $C_{1-3}$alkylS(O)$_2$- (such as methylsulphonyl which is MeS(O)$_2$-), $C_{1-3}$alkylS(O)$_2$-NH- such as methyl-$SO_2$-NH-, $Me_2$N-S(O)$_2$-, $H_2$N-S(O)$_2$-, $-CONH_2$, -CONHMe, $-CO_2H$, cyano (CN), $NMe_2$, t-butoxymethyl, or $C_{1-3}$alkylS(O)$_2$-$CH_2$- such as methyl-$SO_2$-$CH_2$-. More preferably, each $R^6$, independently of any other $R^6$ present, is a fluorine, chlorine, bromine or iodine atom, methyl, ethyl, n-propyl, isopropyl, isobutyl, trifluoromethyl, $-CH_2OH$, methoxy, ethoxy, $C_1$fluoroalkoxy (e.g. trifluoromethoxy or difluoromethoxy), $C_{1-3}$alkylS(O)$_2$- such as methylsulphonyl, $C_{1-3}$alkylS(O)$_2$-NH- such as methyl-$SO_2$-NH-, $Me_2$N-S(O)$_2$-, $H_2$N-S(O)$_2$-, $-CONH_2$, or $C_{1-3}$alkylS(O)$_2$-$CH_2$- such as methyl-$SO_2$-$CH_2$. Still more preferably, each $R^6$, independently of any other $R^6$ present, is a fluorine, chlorine or bromine atom, methyl, ethyl, n-propyl, isopropyl, trifluoromethyl, $-CH_2OH$, methoxy, difluoromethoxy, methylsulphonyl, methyl-$SO_2$-NH- or methyl-$SO_2$-$CH_2$-.

In sub-formula (x), preferably, one, two or three $R^6$ substituents are present in B, D and/or E; so that for example in sub-formula (x), one, two or three $R^6$ substituents are present in the meta- (3- and/or 5-) and/or para- (4-) positions with respect to the $-(CH_2)_n-$ side-chain.

Preferably, $R^5$ has the sub-formula (x), n is 1 and each of A, B, D, E and F is independently CH or $CR^6$; that is $R^5$ has the sub-formula (x) and is optionally substituted benzyl.

In one preferable embodiment, $R^5$ has the sub-formula (x) and is: benzyl, (monoalkyl-phenyl)methyl, [mono(fluoroalkyl)-phenyl]methyl, (monohalo-phenyl)methyl, (monoalkoxy-phenyl)methyl, [mono(fluoroalkoxy)-phenyl]methyl, [mono(N,N-

dimethylamino)-phenyl]methyl, [mono(methyl-$SO_2$-NH-)-phenyl]methyl, [mono(methyl-$SO_2$-)-phenyl]methyl, (dialkyl-phenyl)methyl, (monoalkyl-monohalo-phenyl)methyl, [mono(fluoroalkyl)-monohalo-phenyl]methyl, (dihalo-phenyl)methyl, (dihalo-monoalkyl-phenyl)methyl, [dihalo-mono(hydroxymethyl)-phenyl]methyl, or (dialkoxy-phenyl)methyl such as (3,4-dimethoxy-phenyl)methyl. The substituents can preferably be further defined, as defined in preferable embodiments herein.

In one preferable embodiment, $R^5$ is of sub-formula (x) and is: (monoalkyl-phenyl)methyl, [mono(fluoroalkyl)-phenyl]methyl, (monohalo-phenyl)methyl, (monoalkoxy-phenyl)methyl, [mono(fluoroalkoxy)-phenyl]methyl, [mono(N,N-dimethylamino)-phenyl]methyl, (dialkyl-phenyl)methyl, (monoalkyl-monohalo-phenyl)methyl, (dihalo-phenyl)methyl or (dihalo-monoalkyl-phenyl)methyl or [dihalo-mono(hydroxymethyl)-phenyl]methyl. More preferably, in this embodiment, $R^5$ is:
- (mono$C_{1-3}$alkyl-phenyl)methyl such as (4-$C_{1-3}$alkyl-phenyl)methyl;
- (mono$C_1$fluoroalkyl-phenyl)methyl such as (4-$C_1$fluoroalkyl-phenyl)methyl;
- (mono$C_{1-2}$alkoxy-phenyl)methyl such as (4-$C_{1-2}$alkoxy-phenyl)methyl;
- [mono($C_1$fluoroalkoxy)-phenyl]methyl such as (4-$C_1$fluoroalkoxy-phenyl)methyl;
- (di$C_{1-2}$alkyl-phenyl)methyl or (dimethyl-phenyl)methyl such as (3,4-dimethyl-phenyl)methyl, (2,4-dimethyl-phenyl)methyl, (3,5-dimethyl-phenyl)methyl, (2,3-dimethyl-phenyl)methyl or (2,5-dimethyl-phenyl)methyl; more preferably (3,4-dimethyl-phenyl)methyl or (2,4-dimethyl-phenyl)methyl;
- (mono$C_{1-2}$alkyl-monohalo-phenyl)methyl or (mono$C_{1-2}$alkyl-monochloro-phenyl)methyl such as (4-methyl-3-chloro-phenyl)methyl, (3-methyl-4-chloro-phenyl)methyl, (2-methyl-4-chloro-phenyl)methyl;
- (dihalo-phenyl)methyl such as (2-chloro-4-fluorophenyl)methyl or (2,4-difluoro-phenyl)methyl or (4-bromo-2-fluorophenyl)methyl or preferably (4-chloro-2-fluorophenyl)methyl; for example (dichloro-phenyl)methyl such as (3,4-dichloro-phenyl)methyl or (2,4-dichloro-phenyl)methyl or (2,6-dichloro-phenyl)methyl or preferably (2,3-dichloro-phenyl)methyl;
- (dihalo-mono$C_{1-2}$alkyl-phenyl)methyl e.g. (2,4-dichloro-6-methyl-phenyl)methyl; or
- [dihalo-mono(hydroxymethyl)-phenyl]methyl such as [2,3-dichloro-6-(hydroxymethyl)-phenyl]methyl.

In an alternative preferable embodiment, $R^5$ has the sub-formula (z), and one or preferably none of J, L, M or Q is $CR^6$, and/or $R^9$ is a hydrogen atom (H) or methyl. Preferably r is 1. Preferably, for (z), $R^6$ is independently OH (including any keto tautomer thereof), or more preferably $C_{1-2}$alkyl (e.g. methyl) or $C_1$fluoroalkyl.

Preferred compounds of formula(I) include:
4-{[1-(aminocarbonyl)-4-piperidinyl]amino} -N- [(2,4-dimethylphenyl)methyl] -1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide; and

(cis)- 4-{[4-(aminocarbonyl)cyclohexyl]amino}-*N*-[(3,4-dimethylphenyl)methyl]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide;
or a salt thereof, e.g. a pharmaceutically acceptable salt thereof.

Because of their potential use in medicine, the salts of the compounds of formula (I) are preferably pharmaceutically acceptable. Suitable pharmaceutically acceptable salts can include acid or base addition salts.

A pharmaceutically acceptable acid addition salt can be formed by reaction of a compound of formula (I) with a suitable inorganic or organic acid (such as hydrobromic, hydrochloric, sulfuric, nitric, phosphoric, succinic, maleic, formic, acetic, propionic, fumaric, citric, tartaric, lactic, benzoic, salicylic, glutamaic, aspartic, p-toluenesulfonic, benzenesulfonic, methanesulfonic, ethanesulfonic, naphthalenesulfonic such as 2-naphthalenesulfonic, or hexanoic acid), optionally in a suitable solvent such as an organic solvent, to give the salt which is usually isolated, for example by crystallisation and filtration. A pharmaceutically acceptable acid addition salt of a compound of formula (I) can comprise or be for example a hydrobromide, hydrochloride, sulfate, nitrate, phosphate, succinate, maleate, formate, acetate, propionate, fumarate, citrate, tartrate, lactate, benzoate, salicylate, glutamate, aspartate, p-toluenesulfonate, benzenesulfonate, methanesulfonate, ethanesulfonate, naphthalenesulfonate (e.g. 2- naphthalenesulfonate) or hexanoate salt.

A pharmaceutically acceptable base addition salt can be formed by reaction of a compound of formula (I) with a suitable inorganic or organic base (e.g. triethylamine, ethanolamine, triethanolamine, choline, arginine, lysine or histidine), optionally in a suitable solvent such as an organic solvent, to give the base addition salt which is usually isolated for example by crystallisation and filtration.

Other suitable pharmaceutically acceptable salts include pharmaceutically acceptable metal salts, for example pharmaceutically acceptable alkali-metal or alkaline-earth-metal salts such as sodium, potassium, calcium or magnesium salts; in particular pharmaceutically acceptable metal salts of one or more carboxylic acid moieties that may be present in the the compound of formula (I).

Other non-pharmaceutically acceptable salts, eg. oxalates, may be used, for example in the isolation of compounds of the invention, and are included within the scope of this invention.

The invention includes within its scope all possible stoichiometric and non-stoichiometric forms of the salts of the compounds of formula (I).

Also included within the scope of the invention are all solvates, hydrates and complexes of compounds and salts of the invention.

Certain groups, substituents, compounds or salts included in the present invention may be present as isomers. The present invention includes within its scope all such isomers, including racemates, enantiomers and mixtures thereof.

In the compounds or salts, pharmaceutical compositions, uses, methods of treatment/prophylaxis, methods of preparing, etc. according to the present invention, where a defined isomeric configuration e.g. stereochemical configuration is described or claimed, the invention includes a mixture comprising (a) a major component of the compound or salt which is in the described or claimed configuration, together with (b) one or more minor components of the compound or salt which is/are not in the described or claimed configuration. Preferably, in such a mixture, the major component of the compound or salt which is in the described or claimed configuration represents 70% or more, or 75% or more, more preferably 85% or more, still more preferably 90% or more, yet more preferably 95% or more, yet more preferably 98% or more, of the total amount of compound or salt present in the mixture on a molarity basis.

Certain of the groups, e.g. heteroaromatic ring systems, included in compounds of formula (I) or their salts may exist in one or more tautomeric forms. The present invention includes within its scope all such tautomeric forms, including mixtures.

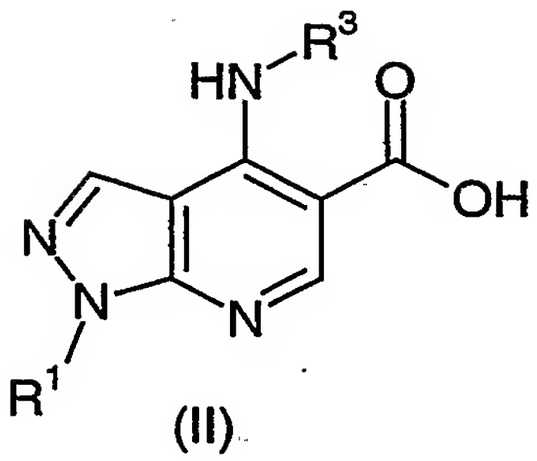
Especially when intended for oral medicinal use, preferred compound(s) of formula (I) are those that have a molecular weight of 1000 or less, for example 800 or less, in particular 650 or less or 600 or less. Molecular weight here refers to that of the unsolvated "free base" compound, that is excluding any molecular weight contributed by any addition salts, solvent (e.g. water) molecules, etc.

**Synthetic Process Routes**

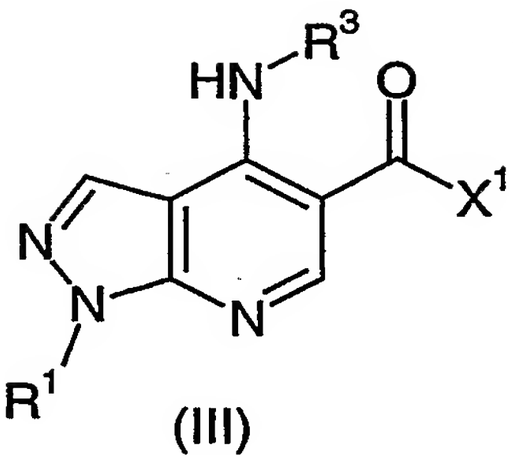
The following processes can be used to make the compounds of formula (I) as hereinbefore defined. Most of the following synthetic processes are exemplified for compounds of Formula (I) wherein $R^2$ is a hydrogen atom (H). However, some or all of these processes can also be used with appropriate modification, e.g. of starting materials and reagents, for making compounds of Formula (I) wherein $R^2$ is methyl.

**Process A**

To form a compound of formula (I), a carboxylic acid of formula (II) can be converted into an activated compound of formula (III) wherein $X^1$ = a leaving group substitutable by an amine (as defined below) and subsequently the activated compound can be reacted with an amine of formula $NHR^4R^5$:

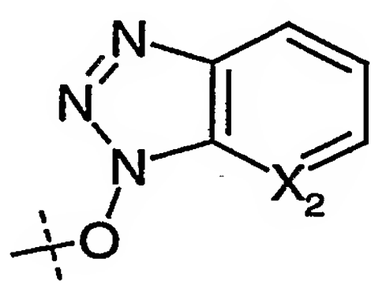


(II)



(III)

For example, the activated compound (the compound of formula (III)) can be the acid chloride. This can be formed from the carboxylic acid (II) e.g. by reaction with thionyl chloride, either in an organic solvent such as chloroform or without solvent. Alternatively, the activated compound (the compound of formula (III)) can be an activated ester wherein the leaving group $X^1$ is



$X_2$ = CH or N

The latter activated compound of formula (III) can be formed from the carboxylic acid (II) either:

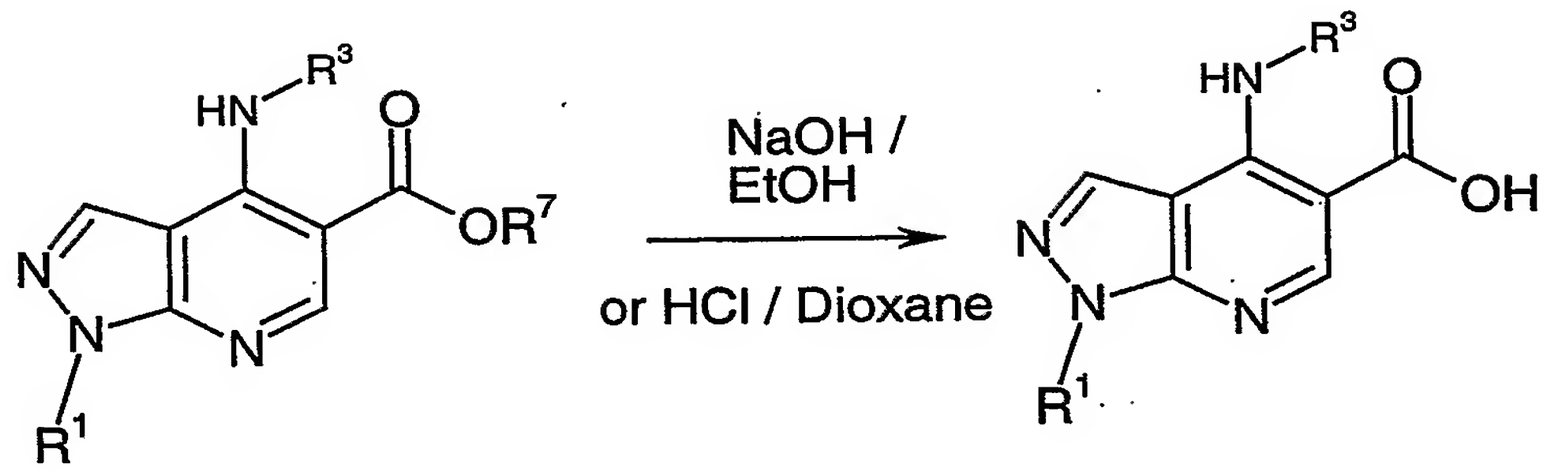
(a) by reaction of the carboxylic acid with a carbodiimide such as EDC (1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide), or a salt thereof e.g. hydrochloride salt, preferably followed by reaction of the resulting product with 1-hydroxybenzotriazole (HOBT); reaction (a) usually being carried out in the presence of a solvent (preferably anhydrous) such as dimethyl formamide (DMF) or acetonitrile and/or preferably under anhydrous conditions and/or usually at room temperature (e.g. about 20 to about 25 °C); or:

(b) by reaction with 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) ,in the presence of a base such as diisopropylethylamine ($^iPr_2NEt$ = DIPEA), and usually in the presence of a solvent such as dimethyl formamide (DMF) or acetonitrile and/or preferably under anhydrous conditions and/or usually at room temperature (e.g. about 20 to about 25 °C).

Compounds of formula (II) can be prepared by hydrolysis of an ester of formula (IV). This procedure preferably involves reaction of (IV) with either:

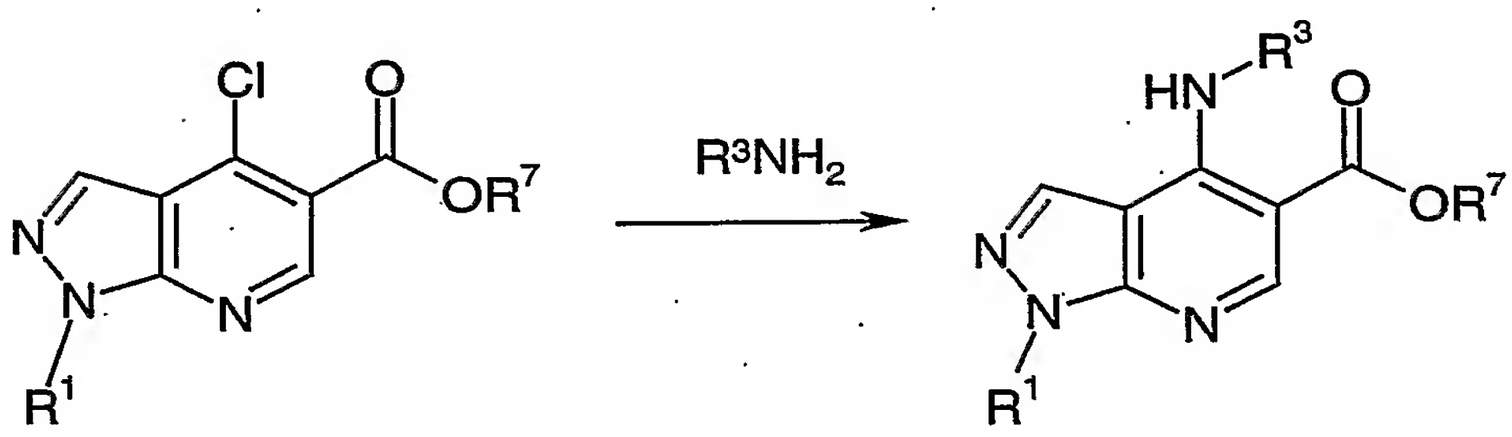
(a) a base such as sodium hydroxide or potassium hydroxide in a solvent e.g. an aqueous solvent such as aqueous ethanol or aqueous dioxane or

(b) an acid such as hydrochloric acid in a solvent e.g. an aqueous solvent such as aqueous dioxane:



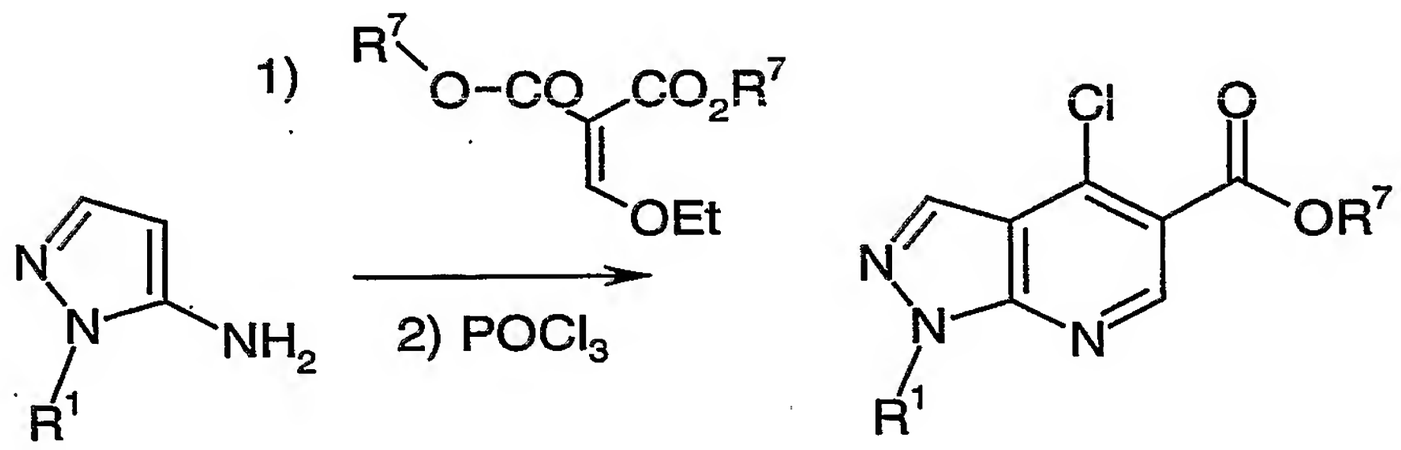

( IV ) ( II )

Compounds of formula (IV) can be prepared according to a method, for example as described by Yu et. al. in *J. Med Chem.*, 2001, 44, 1025-1027, by reaction of a compound of formula (V) with an amine of formula $R^3NH_2$. The reaction is preferably carried out in the presence of a base such as triethylamine or N,N-diisopropylethylamine, and/or in an organic solvent such as ethanol, dioxane or acetonitrile. The reaction may require heating e.g. to ca. 60-100°C, for example ca. 80-90°C:



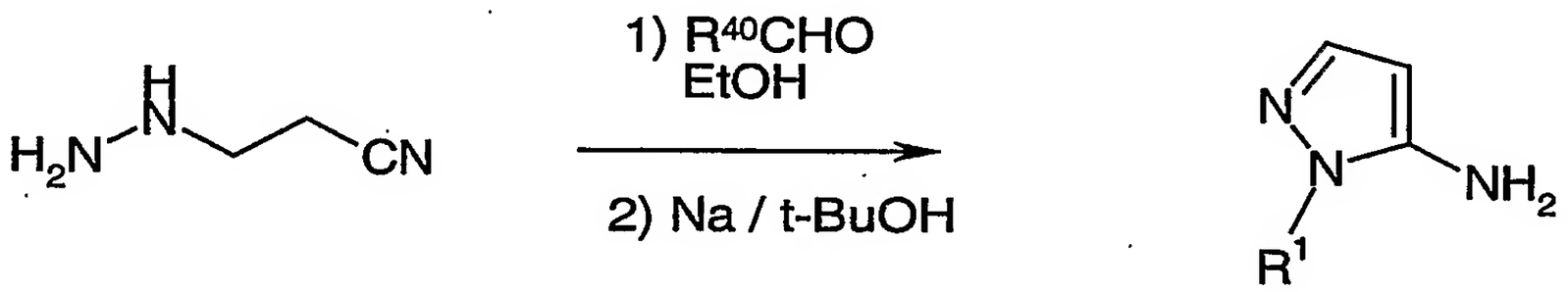

(V) (IV)

Compounds of formula (V) are also described in the above reference and can be prepared by reaction of a compound of formula (VI) with, for example, diethylethoxymethylene malonate (where $R^7$ = Et) with heating, followed by reaction with phosphorous oxychloride, again with heating:




( VI ) ( V )

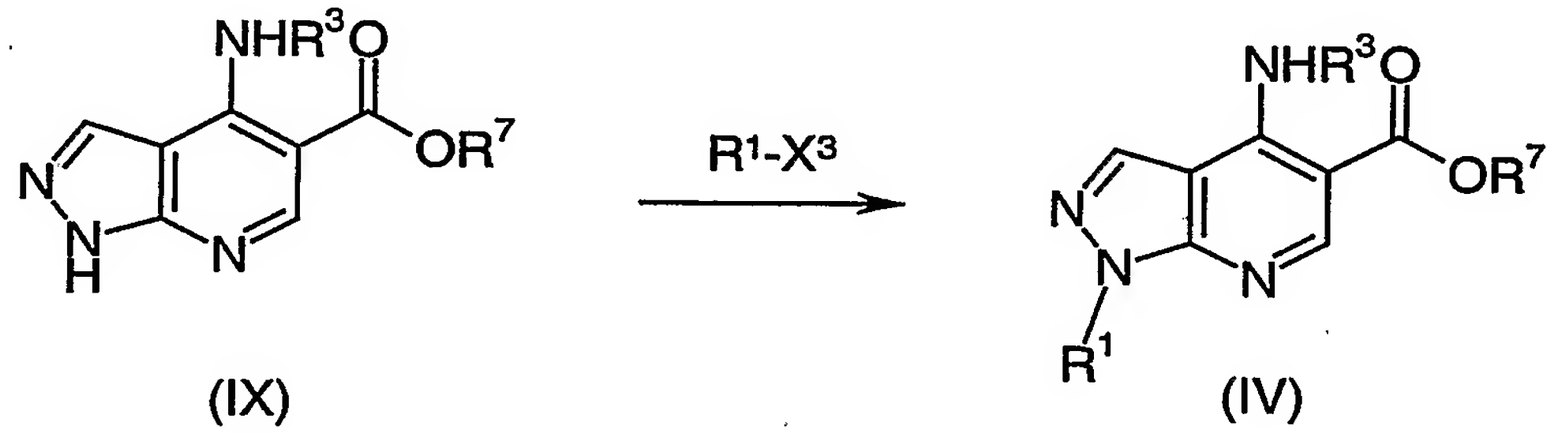
Where the desired amino pyrazole of formula (VI) is not commercially available, preparation can be achieved using methods described by Dorgan et. al. in J. Chem. Soc., Perkin Trans. 1, (4), 938-42; 1980, by reaction of cyanoethylhydrazine with a suitable aldehyde of formula $R^{40}CHO$ in a solvent such as ethanol, with heating, followed by reduction with, for example sodium in a solvent such as t-butanol. $R^{40}$ should be chosen so as to contain one less carbon atom than $R^1$, for example $R^{40}$ = methyl will afford $R^1$ = ethyl.




( VI )

In an alternative embodiment of Process A, the 4-chloro substituent in the compound of formula (V) can be replaced by another halogen atom, such as a bromine atom, or by another suitable leaving group which is displaceable by an amine of formula $R^3NH_2$. The leaving group can, for example, be an alkoxy group $-OR_{35}$ such as $-OC_{1-4}$alkyl (in particular –OEt) or a group $-O\text{-}S(O)_2\text{-}R^{37}$, wherein $R^{37}$ is $C_{1-8}$alkyl (e.g. $C_{1-4}$alkyl or $C_{1-2}$alkyl such as methyl), $C_{1-6}$fluoroalkyl (e.g. $C_{1-4}$fluoroalkyl or $C_{1-2}$fluoroalkyl such as $CF_3$ or $C_4F_9$), or phenyl wherein the phenyl is optionally substituted by one or two of independently $C_{1-2}$alkyl, halogen or $C_{1-2}$alkoxy (such as phenyl or 4-methyl-phenyl). The reaction may be carried out with or without solvent and may require heating.

Compounds of formula (IV) can also be prepared by reaction of a compound of formula (IX) with an alkylating agent of formula $R^1\text{-}X^3$, where $X^3$ is a leaving group displaceable by the 1-position pyrazolopyridine nitrogen atom of the compound of formula (IX):

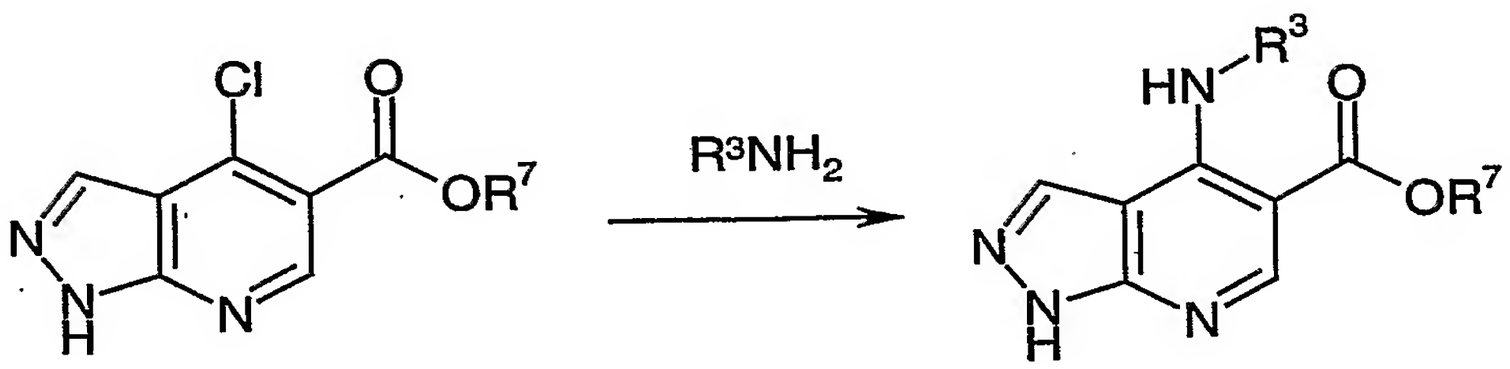



(IX) (IV)

A suitable alkylating agent of formula $R^1\text{-}X^3$ can be used. For example, $X^3$ can be a halogen atom such as a chlorine atom or more preferably a bromine or iodine atom, or $X^3$ can be $-O\text{-}S(O)_2\text{-}R^{36}$ wherein $R^{36}$ is $C_{1-8}$alkyl (e.g. $C_{1-4}$alkyl or $C_{1-2}$alkyl such as

methyl), $C_{1-6}$fluoroalkyl (e.g. $C_{1-4}$fluoroalkyl or $C_{1-2}$fluoroalkyl such as $CF_3$ or $C_4F_9$), or phenyl wherein the phenyl is optionally substituted by one or two of independently $C_{1-2}$alkyl, halogen or $C_{1-2}$alkoxy (such as phenyl or 4-methyl-phenyl). The reaction is preferably carried out in the presence of a base; the base can for example comprise or be potassium carbonate, sodium carbonate, sodium hydride, potassium hydride, or a basic resin or polymer such as polymer-bound 2-tert-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine. The reaction is preferably carried out in the presence of a solvent, e.g. an organic solvent such as DMF; the solvent is preferably anhydrous.

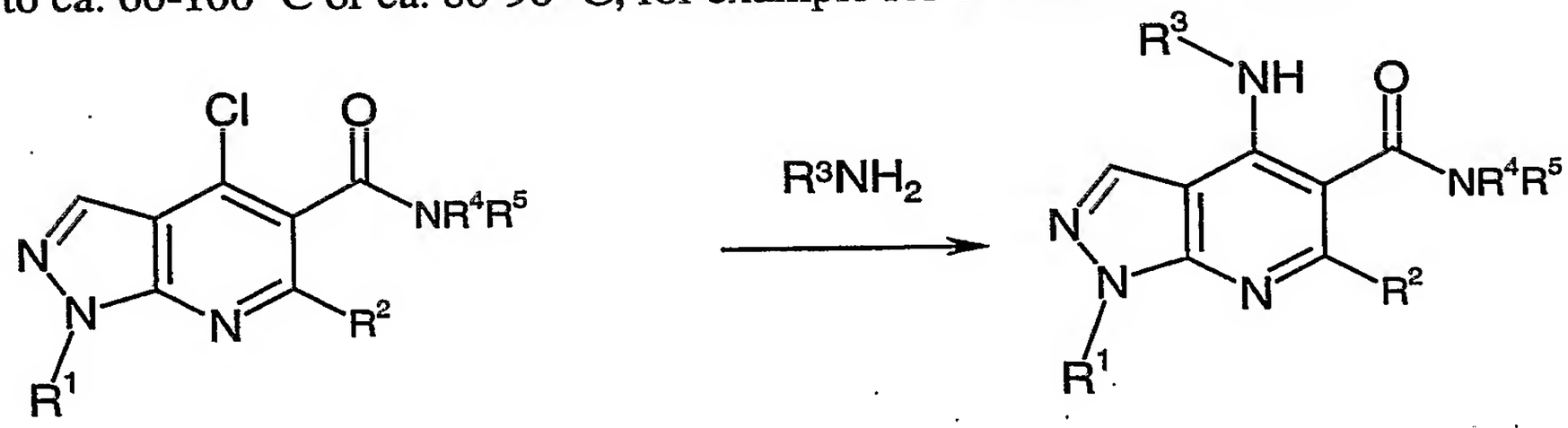
Compounds of formula (IX) can be prepared, using a method analogous to that used for the preparation of compounds (IV), by reaction of a compound of formula (V) ($R^1$ = H) with an amine of formula $R^3NH_2$. The reaction is preferably carried out in the presence of a base such as triethylamine or N,N-diisopropylethylamine, and/or in an organic solvent such as ethanol, dioxane or acetonitrile. The reaction may require heating e.g. to ca. 60-100°C, for example ca. 80-90°C:




(V) (IX)

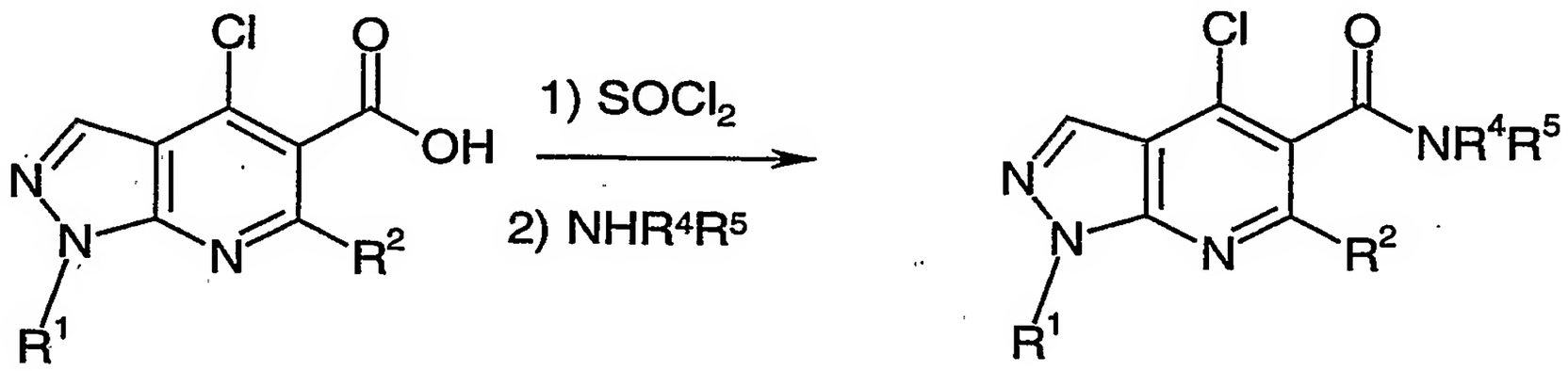
**Process B**

Compounds of formula (I) can be prepared by reaction of a compound of formula (VII) with an amine of formula $R^3NH_2$. The reaction is preferably carried out in the presence of a base, such as triethylamine or N,N-diisopropylethylamine, and/or in an organic solvent such as ethanol, THF, dioxane or acetonitrile. The reaction may require heating, e.g. to ca. 60-100 °C or ca. 80-90 °C, for example for 8-48 or 12-24 hours:



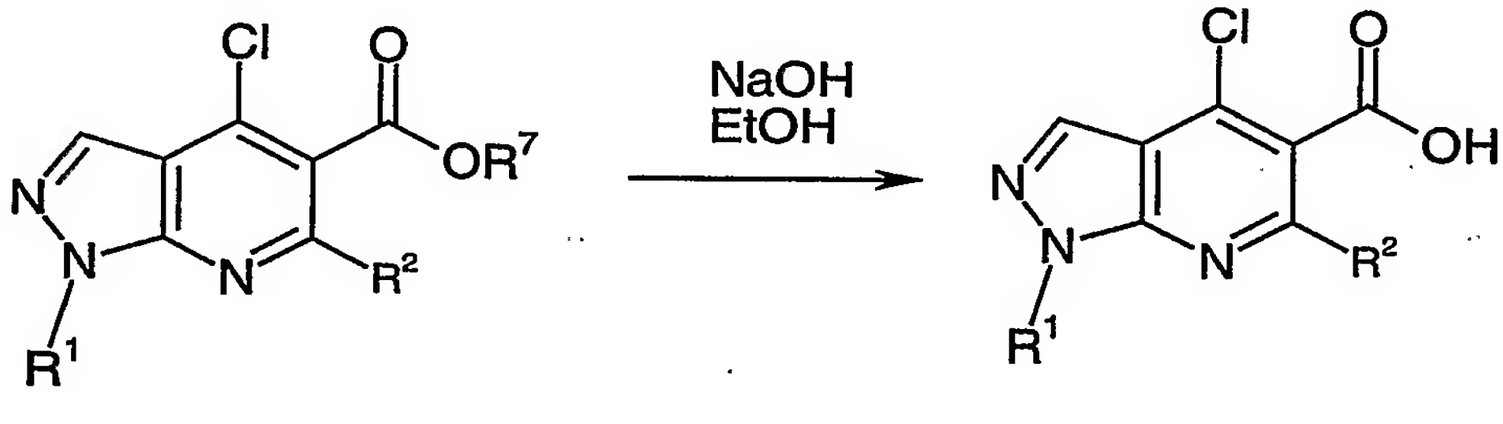

( VII ) ( I )

Compounds of formula (VII) can be prepared in a two step procedure as described by Bare et. al. in *J. Med. Chem.* 1989, 32, 2561-2573. This process involves, first, reaction of a compound of formula (VIII) with thionyl chloride (or another agent suitable for forming an acid chloride from a carboxylic acid), either in an organic solvent such as chloroform or THF, or as a neat solution. This reaction may require heating and the thus-formed intermediate may or may not be isolated. Step two involves reaction with an amine of formula $ArCR^5R^6NHR^4$, in an organic solvent such as THF or chloroform and may also involve the use of a base such as triethylamine or diisopropylethylamine:




( VIII ) ( VII )

Compounds of formula (VIII) can be prepared by hydrolysis of an ester of formula (V) according to the method described by Yu et. al. in *J. Med Chem.*, 2001, 44, 1025-1027. This procedure preferably involves reaction with a base such as sodium hydroxide or potassium hydroxide in a solvent e.g. an aqueous solvent such as aqueous ethanol or aqueous dioxane:




( V ) ( VIII )

In an alternative embodiment of Process B, the 4-chloro substituent in the compound of formula (IV) can be replaced by another halogen atom, such as a bromine atom.

**Process C:** *Conversion of one compound of formula (I)-(IV) or salt thereof into another compound of formula (I)-(IV) or salt thereof*

One compound of formula (I)-(IV) or salt thereof can be converted into another compound of formula (I)-(IV) or salt thereof. This conversion preferably comprises or is one or more of the following processes C1 to C6:

C1. An oxidation process. For example, the oxidation process can comprise or be oxidation of an alcohol to a ketone (e.g. using Jones reagent) or oxidation of an alcohol or a ketone to a carboxylic acid.

C2. A reduction process, for example reduction of a ketone or a carboxylic acid to an alcohol.

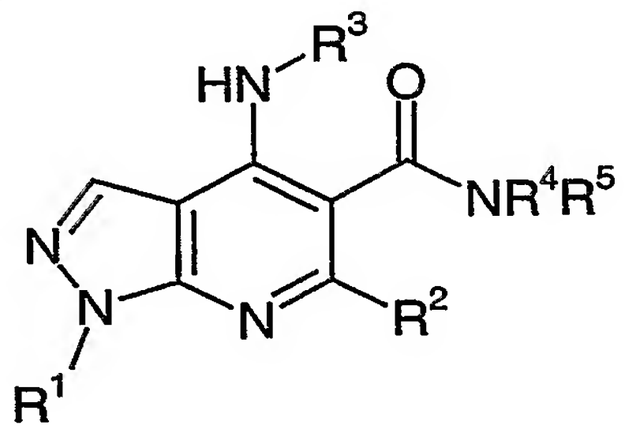
C3. Alkylation, for example alkylation of an amine or of a hydroxy group.

C4. Hydrolysis, e.g. hydrolysis of an ester to the corresponding carboxylic acid or salt thereof.

C5. Deprotection, e.g. deprotection (e.g. deacylation or t-butyloxycarbonyl (BOC) removal) of an amine group.

C6. Formation of an ester or amide, for example from the corresponding carboxylic acid.

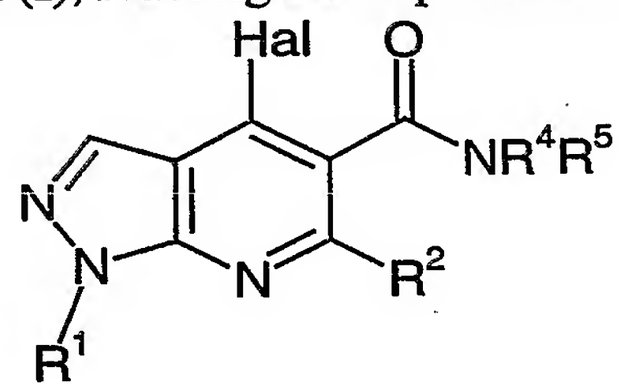
The present invention therefore also provides a method of preparing a compound of formula (I) or a salt thereof:



(I)

wherein $R^1$, $R^2$, $R^3$, $R^4$ and $R^5$ are as defined herein, the method comprising :

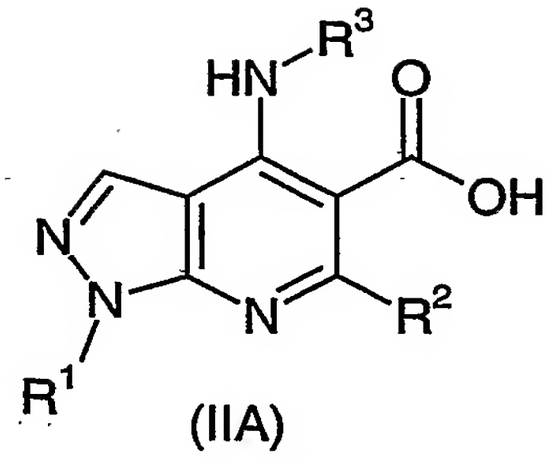
(a) for a compound of formula (I), reacting a compound of formula (VIIA) :



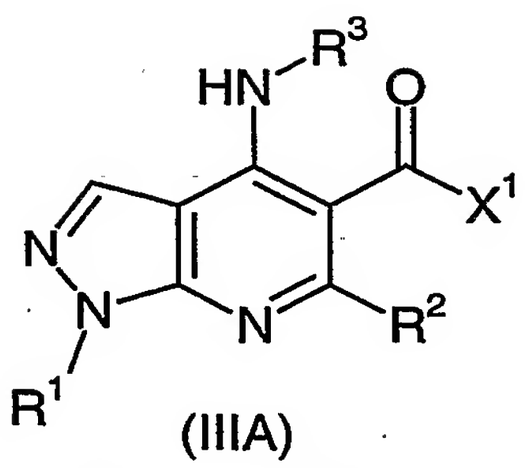
(VIIA)

wherein Hal is a halogen atom (such as a bromine atom or preferably a chlorine atom), with an amine of formula $R^3NH_2$, or

(b) converting a compound of formula (IIA) into an activated compound of formula (IIIA) wherein $X^1$ = a leaving group substitutable by an amine:



(IIA)



(IIIA)

and subsequent reaction of the activated compound of formula (IIIA) with an amine of formula $R^4R^5NH$;

and thereafter, and if so desired, converting one compound of formula (I) or salt thereof into another compound of formula (I) or salt thereof;

and optionally converting the compound of formula (I) into a salt thereof e.g. a pharmaceutically acceptable salt thereof.

The present invention also provides: (g) a method of preparing a pharmaceutically acceptable salt of a compound of formula (I) comprising conversion of the compound of formula (I) or a salt thereof into the desired pharmaceutically acceptable salt thereof.
The present invention also provides a compound of formula (I) or a salt thereof, prepared by a method as defined herein.

The present invention also provides a compound of formula (I) or a pharmaceutically acceptable salt thereof for use as an active therapeutic substance in a mammal such as a human. The compound or salt can be for use in the treatment and/or prophylaxis of any of the diseases / conditions described herein (e.g. for use in the treatment and/or prophylaxis of an inflammatory and/or allergic disease in a mammal) and/or for use as a phosphodiesterase inhibitor e.g. for use as a phosphodiesterase 4 (PDE4) inhibitor.
"Therapy" may include treatment and/or prophylaxis.

Also provided is the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof in the manufacture of a medicament (e.g. pharmaceutical composition) for the treatment and/or prophylaxis of any of the diseases / conditions described herein in a mammal such as a human, e.g. for the treatment and/or prophylaxis of an inflammatory and/or allergic disease in a mammal such as a human.

Also provided is a method of treatment and/or prophylaxis of any of the diseases / conditions described herein in a mammal (e.g. human) in need thereof, e.g. a method of treatment and/or prophylaxis of an inflammatory and/or allergic disease in a mammal (e.g. human) in need thereof, which method comprises administering to the mammal (e.g.

human) a therapeutically effective amount of a compound of formula (I) as herein defined or a pharmaceutically acceptable salt thereof.

Phosphodiesterase 4 inhibitors are thought to be useful in the treatment and/or prophylaxis of a variety of diseases / conditions, especially inflammatory and/or allergic diseases, in mammals such as humans, for example: asthma, chronic obstructive pulmonary disease (COPD) (e.g. chronic bronchitis and/or emphysema), atopic dermatitis, urticaria, allergic rhinitis, allergic conjunctivitis, vernal conjunctivitis, eosinophilic granuloma, psoriasis, rheumatoid arthritis, septic shock, ulcerative colitis, Crohn's disease, reperfusion injury of the myocardium and brain, chronic glomerulonephritis, endotoxic shock, adult respiratory distress syndrome, multiple sclerosis, cognitive impairment (e.g. in a neurological disorder such as Alzheimer's disease), depression, or pain. Ulcerative colitis and/or Crohn's disease are collectively often referred to as inflammatory bowel disease.

In the treatment and/or prophylaxis, the inflammatory and/or allergic disease is preferably chronic obstructive pulmonary disease (COPD), asthma, rheumatoid arthritis or allergic rhinitis in a mammal (e.g. human). More preferably, the treatment and/or prophylaxis is of COPD or asthma in a mammal (e.g. human).

PDE4 inhibitors are thought to be effective in the treatment of asthma (e.g. see M.A.Giembycz, *Drugs*, Feb. 2000, 59(2), 193-212; Z. Huang et al., *Current Opinion in Chemical Biology*, 2001, 5: 432-438; H.J.Dyke et al., *Expert Opinion on Investigational Drugs*, January 2002, 11(1), 1-13; C.Burnouf et al., *Current Pharmaceutical Design*, 2002, 8(14), 1255-1296; A.M.Doherty, *Current Opinion Chem. Biol.*, 1999, 3(4), 466-473; and references cited in the aforementioned publications).

PDE4 inhibitors are thought to be effective in the treatment of COPD. For example, see S.L. Wolda, *Emerging Drugs*, 2000, 5(3), 309-319; Z. Huang et al., *Current Opinion in Chemical Biology*, 2001, 5: 432-438; H.J.Dyke et al., *Expert Opinion on Investigational Drugs*, January 2002, 11(1), 1-13; C.Burnouf et al., *Current Pharmaceutical Design*, 2002, 8(14), 1255-1296; A.M.Doherty, *Current Opinion Chem. Biol.*, 1999, 3(4), 466-473; and references cited in the aforementioned publications; and G. Krishna et al., *Expert Opinion on Investigational Drugs*, 2004, 13(3), 255-267 (see especially pp. 259-261 and refs. 102-111 and 201 therein). COPD is often characterised by the presence of airflow obstruction due to chronic bronchitis and/or emphysema (e.g., see S.L. Wolda, *Emerging Drugs*, 2000, 5(3), 309-319).

PDE4 inhibitors are thought to be effective in the treatment of allergic rhinitis (e.g. see B.M. Schmidt et al., *J. Allergy & Clinical Immunology*, 108(4), 2001, 530-536).

PDE4 inhibitors are thought to be effective in the treatment of rheumatoid arthritis and multiple sclerosis (e.g. see H.J.Dyke et al., *Expert Opinion on Investigational Drugs*, January 2002, 11(1), 1-13; C.Burnouf et al., *Current Pharmaceutical Design*, 2002, 8(14), 1255-1296; and A.M.Doherty, *Current Opinion Chem. Biol.*, 1999, 3(4), 466-473; and references cited in these publications). See e.g. A.M.Doherty, *Current Opinion Chem. Biol.*, 1999, 3(4), 466-473 and references cited therein for atopic dermatitis use.

PDE4 inhibitors have been suggested as having analgesic properties and thus being effective in the treatment of pain (A.Kumar et al., *Indian J. Exp. Biol.*, 2000, 38(1), 26-30).

In the invention, the treatment and/or prophylaxis can be of cognitive impairment e.g. cognitive impairment in a neurological disorder such as Alzheimer's disease. For example, the treatment and/or prophylaxis can comprise cognitive enhancement e.g. in a neurological disorder. See for example: H.T.Zhang et al. in: *Psychopharmacology*, June 2000, 150(3), 311-316 and *Neuropsychopharmacology*, 2000, 23(2), 198-204; and T. Egawa et al., *Japanese J. Pharmacol.*, 1997, 75(3), 275-81.

PDE4 inhibitors such as rolipram have been suggested as having antidepressant properties (e.g. J. Zhu et al., *CNS Drug Reviews*, 2001, 7(4), 387-398; O'Donnell, *Expert Opinion on Investigational Drugs*, 2000, 9(3), 621-625; and H.T. Zhang et al., *Neuropsychopharmacology*, October 2002, 27(4), 587-595).

**Pharmaceutical compositions and dosing**

For use in medicine, the compounds of the present invention are usually administered as a pharmaceutical composition.

The present invention therefore provides in a further aspect a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable carriers and/or excipients.

The pharmaceutical composition can be for use in the treatment and/or prophylaxis of any of the conditions described herein.

The invention also provides a method of preparing a pharmaceutical composition comprising a compound of formula (I), as herein defined, or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable carriers and/or excipients, he method comprising mixing the compound or salt with the one or more pharmaceutically acceptable carriers and/or excipients.

The invention also provides a pharmaceutical composition prepared by said method.

The compounds of formula (I) and/or the pharmaceutical composition may be administered, for example, by oral, parenteral (e.g. intravenous, subcutaneous, or intramuscular), inhaled or nasal administration. Accordingly , the pharmaceutical composition is preferably suitable for oral, parenteral (e.g. intravenous, subcutaneous, or intramuscular), inhaled or nasal administration. More preferably, the pharmaceutical composition is suitable for inhaled or oral administration, e.g. to a mammal such as a human. Inhaled administration involves topical administration to the lung e.g. by aerosol or dry powder composition. Oral administration to a human is most preferred.

A pharmaceutical composition suitable for oral administration can be liquid or solid; for example it can be a syrup, suspension or emulsion, a tablet, a capsule or a lozenge.

A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt in a suitable pharmaceutically acceptable liquid carrier(s), for example an aqueous solvent such as water, ethanol or glycerine, or a non-aqueous solvent, such as polyethylene glycol or an oil. The formulation may also contain a suspending agent, preservative, flavouring and/or colouring agent.

A pharmaceutical composition suitable for oral administration being a tablet can comprise one or more pharmaceutically acceptable carriers and/or excipients suitable for preparing tablet formulations. The carrier can for example be or include lactose, cellulose (for example microcrystalline cellulose), or mannitol. The tablet can also or instead contain one or more pharmaceutically acceptable excipients, for example a binding agent such as hydroxypropylmethylcellulose or povidone (polyvinylpyrollidone), a lubricant e.g. an alkaline earth metal stearate such as magnesium stearate, and/or a tablet disintegrant such as sodium starch glycollate, croscarmellose sodium, or crospovidone (cross-linked polyvinylpyrollidone). The pharmaceutical composition being a tablet can be prepared by a method comprising the steps of: (i) mixing the compound of formula (I), as herein defined, or a pharmaceutically acceptable salt thereof, with the one or more pharmaceutically acceptable carriers and/or excipients, (ii) compressing the resulting mixture (which is usually in powder form) into tablets, and (iii) optionally coating the tablet with a tablet film-coating material.

A pharmaceutical composition suitable for oral administration being a capsule can be prepared using encapsulation procedures. For example, pellets or powder containing the active ingredient can be prepared using a suitable pharmaceutically acceptable carrier and then filled into a hard gelatin capsule. Alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutically acceptable carrier, for example an aqueous gum or an oil and the dispersion or suspension then filled into a soft gelatin capsule.

Preferably the composition is in unit dose form such as a tablet or capsule for oral administration, e.g. for oral administration to a human.

A parenteral composition can comprise a solution or suspension of the compound or pharmaceutically acceptable salt in a sterile aqueous carrier or parenterally acceptable oil. Alternatively, the solution can be lyophilised; the lyophilised parenteral pharmaceutical composition can be reconstituted with a suitable solvent just prior to administration.

Compositions for nasal or inhaled administration may conveniently be formulated as aerosols, drops, gels or dry powders.

Aerosol formulations, e.g. for inhaled administration, can comprise a solution or fine suspension of the active substance in a pharmaceutically acceptable aqueous or non-aqueous solvent. Aerosol formulations can be presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomising device or inhaler. Alternatively the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve (metered dose inhaler) which is intended for disposal once the contents of the container have been exhausted.

Where the dosage form comprises an aerosol dispenser, it preferably contains a suitable propellant under pressure such as compressed air, carbon dioxide, or an organic propellant such as a chlorofluorocarbon (CFC) or hydrofluorocarbon (HFC). Suitable CFC propellants include dichlorodifluoromethane, trichlorofluoromethane and dichlorotetrafluoroethane. Suitable HFC propellants include 1,1,1,2,3,3,3-heptafluoropropane and 1,1,1,2-tetrafluoroethane. The aerosol dosage forms can also take the form of a pump-atomiser.

For pharmaceutical compositions suitable and/or adapted for inhaled administration, it is preferred that the compound or salt of formula (I) is in a particle-size-reduced form, and more preferably the size-reduced form is obtained or obtainable by micronisation. Micronisation usually involves subjecting the compound/salt to collisional and/or abrasional forces in a fast-flowing circular or spiral/vortex-shaped airstream often including a cyclone component. The preferable particle size of the size-reduced (e.g. micronised) compound or salt is defined by a D50 value of about 0.5 to about 10 microns, e.g. about 1 to about 7 microns (e.g. as measured using laser diffraction). For example, it is preferable for the compound or salt of formula (I) to have a particle size defined by: a D10 of about 0.3 to about 3 microns (e.g. about 0.5 to about 2 microns, or about 1 micron), and/or a D50 of about 0.5 to about 10 microns or about 1 to about 7 microns (e.g. about 2 to about 5 microns or about 2 to about 4 microns), and/or a D90 of about 1 to about 30 microns or about 2 to about 20 microns or about 3 to about 15 microns (e.g. about 5 to about 15 microns or about 5 to about 10 microns); for example as measured using laser diffraction.

In particle size measurements, D90, D50 and D10 respectively mean that 90%, 50% and 10% of the material is less than the micron size specified. D50 is the median particle size. DV90, DV50 and DV10 respectively mean that 90%, 50% and 10% by volume of the material is less than the micron size specified. DM90, DM50 and DM10 respectively mean that 90%, 50% and 10% by weight of the material is less than the micron size specified.

Laser diffraction measurement of particle size can use a dry method (wherein a suspension of the compound/salt in an airflow crosses the laser beam) or a wet method [wherein a suspension of the compound/salt in a liquid dispersing medium, such as isooctane or (e.g. if compound is soluble in isooctane) 0.1% Tween 80 in water, crosses the laser beam]. With laser diffraction, particle size is preferably calculated using the Fraunhofer calculation; and/or preferably a Malvern Mastersizer or Sympatec apparatus is used for measurement. For example, particle size measurement and/or analysis by laser diffraction can use any or all of (preferably all of) the following: a Malvern Mastersizer longbed version, a dispersing medium of 0.1% Tween 80 in water, a stir rate of ca. 1500 rpm, ca. 3 mins sonification prior to final dispersion and analysis, a 300 RF (Reverse Fourier) lens, and/or the Fraunhofer calculation with Malvern software.

Optionally, in particular for dry powder inhalable compositions, a pharmaceutical composition for inhaled administration can be incorporated into a plurality of sealed dose containers (e.g. containing the dry powder composition) mounted longitudinally in a strip or ribbon inside a suitable inhalation device. The container is rupturable or peel-openable on demand and the dose, e.g. of the dry powder composition, can be administered by inhalation via a device such as the DISKUS $^{TM}$ device, marketed by GlaxoSmithKline. The DISKUS $^{TM}$ inhalation device is usually substantially as described in GB 2,242,134 A. In such device at least one container for the pharmaceutical composition in powder form (the at least one container preferably being a plurality of sealed dose containers mounted longitudinally in a strip or ribbon) is defined between two members peelably secured to one another; the device comprises: means defining an opening station for the said at least one container; means for peeling the members apart at the opening station to open the container; and an outlet, communicating with the opened container, through which a user can inhale the pharmaceutical composition in powder form from the opened container.

Preferably the composition is in unit dose form such as a tablet or capsule for oral administration, e.g. for oral administration to a human.

In the pharmaceutical composition, a or each dosage unit for oral or parenteral administration preferably contains from 0.01 to 3000 mg, more preferably 0.5 to 1000 mg, of a compound of the formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base. A or each dosage unit for nasal or inhaled administration

preferably contains from 0.001 to 50 mg, more preferably 0.01 to 5 mg, of a compound of the formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base.

A pharmaceutically acceptable compound or salt of the invention is preferably administered to a mammal (e.g. human) in a daily oral or parenteral dose of 0.001 mg to 50 mg per kg body weight per day (mg/kg/day), for example 0.01 to 20 mg/kg/day or 0.03 to 10 mg/kg/day or 0.1 to 2 mg/kg/day, of the compound of the formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base.

A pharmaceutically acceptable compound or salt of the invention is preferably administered to a mammal (e.g. human) in a daily nasal or inhaled dose of: 0.0001 to 5 mg/kg/day or 0.0001 to 1 mg/kg/day, e.g. 0.001 to 1 mg/kg/day or 0.001 to 0.3 mg/kg/day or 0.001 to 0.1 mg/kg/day or 0.005 to 0.3 mg/kg/day, of the compound of the formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base.

The pharmaceutically acceptable compounds or salts of the invention is preferably administered in a daily dose (for an adult patient) of, for example, an oral or parenteral dose of 0.01 mg to 3000 mg per day or 0.5 to 1000 mg per day e.g. 2 to 500 mg per day, or a nasal or inhaled dose of 0.001 to 300 mg per day or 0.001 to 50 mg per day or 0.01 to 30 mg per day or 0.01 to 5 mg per day or 0.02 to 2 mg per day, of the compound of the formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base.

The compounds, salts and/or pharmaceutical compositions according to the invention may also be used in combination with another therapeutically active agent, for example, a $\beta_2$ adrenoreceptor agonist, an anti-histamine, an anti-allergic or an anti-inflammatory agent.

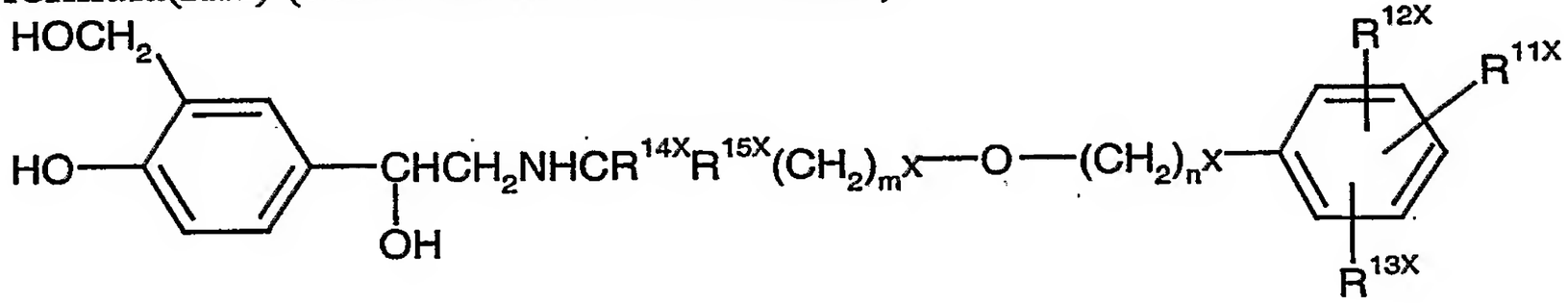
The invention thus provides, in a further aspect, a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof together with another therapeutically active agent, for example, a $\beta_2$-adrenoreceptor agonist, an anti-histamine, an anti-allergic, an anti-inflammatory agent or an antiinfective agent.

Preferably, the $\beta_2$-adrenoreceptor agonist is salmeterol (e.g. as racemate or a single enantiomer such as the R-enantiomer), salbutamol, formoterol, salmefamol, fenoterol or terbutaline, or a salt thereof (e.g. pharmaceutically acceptable salt thereof), for example the xinafoate salt of salmeterol, the sulphate salt or free base of salbutamol or the fumarate salt of formoterol. Long-acting $\beta_2$-adrenoreceptor agonists are preferred, especially those having a therapeutic effect over a 12-24 hour period such as salmeterol or formoterol. Preferably, the $\beta_2$-adrenoreceptor agonist is for inhaled administration, e.g. once per day and/or for simultaneous inhaled administration; and more preferably the $\beta_2$-adrenoreceptor agonist is in particle-size-reduced form e.g. as defined herein. Preferably, the $\beta_2$-adrenoreceptor agonist combination is for treatment and/or

prophylaxis of COPD or asthma. Salmeterol or a pharmaceutically acceptable salt thereof, e.g. salmeterol xinofoate, is preferably administered to humans at an inhaled dose of 25 to 50 micrograms twice per day (measured as the free base). The combination with a $\beta_2$-adrenoreceptor agonist can be as described in WO 00/12078.

Preferred long acting $\beta_2$-adrenoreceptor agonists include those described in WO 02/066422A, WO 03/024439, WO 02/070490 and WO 02/076933.

Especially preferred long-acting $\beta_2$-adrenoreceptor agonists include compounds of formula(XX) (described in WO 02/066422):



(XX)

or a salt or solvate thereof, wherein in formula (XX):
$m^X$ is an integer of from 2 to 8;
$n^X$ is an integer of from 3 to 11,
with the proviso that $m^X + n^X$ is 5 to 19,
$R^{11X}$ is $-XSO_2NR^{16X}R^{17X}$ wherein X is $-(CH_2)_{p^x}-$ or $C_{2-6}$ alkenylene;
$R^{16X}$ and $R^{17X}$ are independently selected from hydrogen, $C_{1-6}$alkyl, $C_{3-7}$cycloalkyl, $C(O)NR^{18X}R^{19X}$, phenyl, and phenyl ($C_{1-4}$alkyl)-,
or $R^{16X}$ and $R^{17X}$, together with the nitrogen to which they are bonded, form a 5-, 6-, or 7-membered nitrogen containing ring, and $R^{16X}$ and $R^{17X}$ are each optionally substituted by one or two groups selected from halo, $C_{1-6}$alkyl, $C_{1-6}$haloalkyl, $C_{1-6}$alkoxy, hydroxy-substituted $C_{1-6}$alkoxy, $-CO_2R^{18X}$, $-SO_2NR^{18X}R^{19X}$, $-CONR^{18X}R^{19X}$, $-NR^{18X}C(O)R^{19X}$, or a 5-, 6- or 7-membered heterocylic ring;
$R^{18X}$ and $R^{19X}$ are independently selected from hydrogen, $C_{1-6}$alkyl, $C_{3-6}$cycloalkyl, phenyl, and phenyl ($C_{1-4}$alkyl)-; and
$p^X$ is an integer of from 0 to 6, preferably from 0 to 4;
$R^{12X}$ and $R^{13X}$ are independently selected from hydrogen, $C_{1-6}$alkyl, $C_{1-6}$alkoxy, halo, phenyl, and $C_{1-6}$haloalkyl; and
$R^{14X}$ and $R^{15X}$ are independently selected from hydrogen and $C_{1-4}$alkyl with the proviso that the total number of carbon atoms in $R^{14X}$ and $R^{15X}$ is not more than 4.

Preferred $\beta_2$-adrenoreceptor agonists disclosed in WO 02/066422 include:
3-(4-{[6-({(2R)-2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)hexyl]oxy}butyl)benzenesulfonamide and
3-(3-{[7-({(2R)-2-hydroxy-2-[4-hydroxy-3-hydroxymethyl)phenyl]ethyl}-amino)heptyl]oxy}propyl)benzenesulfonamide.

A preferred $\beta_2$-adrenoreceptor agonist disclosed in WO 03/024439 is: 4-{(1*R*)-2-[(6-{2-[(2,6-dichlorobenzyl)oxy]ethoxy}hexyl)amino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol.

A combination of a compound of formula (I) or salt together with an anti-histamine is preferably for oral administration (e.g. as a combined composition such as a combined tablet), and can be for treatment and/or prophylaxis of allergic rhinitis. Examples of anti-histamines include methapyrilene, or H1 antagonists such as cetirizine, loratadine (e.g. Clarityn $^{TM}$), desloratadine (e.g. Clarinex $^{TM}$) or fexofenadine (e.g. Allegra $^{TM}$).

The invention also provides, in a further aspect, a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof together with an anticholinergic compound, e.g. a muscarinic (M) receptor antagonist in particular an $M_1$, $M_2$, $M_1/M_2$, or $M_3$ receptor antagonist, more preferably a $M_3$ receptor antagonist, still more preferably a $M_3$ receptor antagonist which selectively antagonises (e.g. antagonises 10 times or more strongly) the $M_3$ receptor over the $M_1$ and/or $M_2$ receptor. For combinations of anticholinergic compounds / muscarinic (M) receptor antagonist with PDE4 inhibitors, see for example WO 03/011274 A2 and WO 02/069945 A2 / US 2002/0193393 A1 and US 2002/052312 A1, and some or all of these publications give examples of anticholinergic compounds / muscarinic (M) receptor antagonists which may be used with the compounds of formula (I) or salts, and/or suitable pharmaceutical compositions. For example, the muscarinic receptor antagonist can comprise or be an ipratropium salt (e.g. ipratropium bromide), an oxitropium salt (e.g. oxitropium bromide), or more preferably a tiotropium salt (e.g. tiotropium bromide); see e.g. EP 418 716 A1 for tiotropium.

The anticholinergic compound or muscarinic (M) receptor antagonist, e.g. $M_3$ receptor antagonist, is preferably for inhaled administration, more preferably in particle-size-reduced form e.g. as defined herein. More preferably, both the muscarinic (M) receptor antagonist and the compound of formula (I) or the pharmaceutically acceptable salt thereof are for inhaled administration. Preferably, the anticholinergic compound or muscarinic receptor antagonist and the compound of formula (I) or salt are for simultaneous administration. The muscarinic receptor antagonist combination is preferably for treatment and/or prophylaxis of COPD.

Other suitable combinations include, for example, a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof together with another anti-inflammatory agent such as an anti-inflammatory corticosteroid; or a non-steroidal anti-inflammatory drug (NSAID) such as a leukotriene antagonist (e.g. montelukast), an iNOS inhibitor, a tryptase inhibitor, a elastase inhibitor, a beta-2 integrin antagonist, a adenosine 2a agonist, a CCR3 antagonist, or a 5-lipoxogenase inhibitor; or an

antiinfective agent (e.g. an antibiotic or an antiviral). An iNOS inhibitor is preferably for oral administration. Suitable iNOS inhibitors (inducible nitric oxide synthase inhibitors) include those disclosed in WO 93/13055, WO 98/30537, WO 02/50021, WO 95/34534 and WO 99/62875. Suitable CCR3 inhibitors include those disclosed in WO 02/26722.

In a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof together with an anti-inflammatory corticosteroid (which is preferably for treatment and/or prophylaxis of asthma, COPD or allergic rhinitis), then preferably the anti-inflammatory corticosteroid is fluticasone, fluticasone propionate (e.g. see US patent 4,335,121), beclomethasone, beclomethasone 17-propionate ester, beclomethasone 17,21-dipropionate ester, dexamethasone or an ester thereof, mometasone or an ester thereof, ciclesonide, budesonide, flunisolide, or a compound as described in WO 02/12266 A1 (e.g. as claimed in any of claims 1 to 22 therein), or a pharmaceutically acceptable salt of any of the above. If the anti-inflammatory corticosteroid is a compound as described in WO 02/12266 A1, then preferably it is Example 1 therein {which is 6α,9α-difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester} or Example 41 therein {which is 6α,9α-difluoro-11β-hydroxy-16α-methyl-17α-[(4-methyl-1,3-thiazole-5-carbonyl)oxy]-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester}, or a pharmaceutically acceptable salt thereof. The anti-inflammatory corticosteroid is preferably for intranasal or inhaled administration. Fluticasone propionate is preferred and is preferably for inhaled administration to a human either (a) at a dose of 250 micrograms once per day or (b) at a dose of 50 to 250 micrograms twice per day.

Also provided is a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof together with $\beta_2$-adrenoreceptor agonist and an anti-inflammatory corticosteroid, for example as described in WO 03/030939 A1. Preferably this combination is for treatment and/or prophylaxis of asthma, COPD or allergic rhinitis. The $\beta_2$-adrenoreceptor agonist and/or the anti-inflammatory corticosteroid can be as described above and/or as described in WO 03/030939 A1. Most preferably, in this "triple" combination, the $\beta_2$-adrenoreceptor agonist is salmeterol or a pharmaceutically acceptable salt thereof (e.g. salmeterol xinafoate) and the anti-inflammatory corticosteroid is fluticasone propionate.
The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical composition and thus a pharmaceutical composition comprising a combination as defined above together with one or more pharmaceutically acceptable carriers and/or excipients represent a further aspect of the invention.

The individual compounds of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical composition.

In one embodiment, the combination as defined herein can be for simultaneous inhaled administration and is disposed in a combination inhalation device. Such a combination inhalation device is another aspect of the invention. Such a combination inhalation device can comprise a combined pharmaceutical composition for simultaneous inhaled administration (e.g. dry powder composition), the composition comprising all the individual compounds of the combination, and the composition being incorporated into a plurality of sealed dose containers mounted longitudinally in a strip or ribbon inside the inhalation device, the containers being rupturable or peel-openable on demand; for example such inhalation device can be substantially as described in GB 2,242,134 A (DISKUS $^{TM}$) and/or as described above. Alternatively, the combination inhalation device can be such that the individual compounds of the combination are administrable simultaneously but are stored separately (or wholly or partly stored separately for triple combinations), e.g. in separate pharmaceutical compositions, for example as described in PCT/EP03/00598 filed on 22 January 2003, published as WO 03/061743 (e.g. as described in the claims thereof e.g. claim 1).

The invention also provides a method of preparing a combination as defined herein, the method comprising either

(a) preparing a separate pharmaceutical composition for administration of the individual compounds of the combination either sequentially or simultaneously, or

(b) preparing a combined pharmaceutical composition for administration of the individual compounds of the combination simultaneously,

wherein the pharmaceutical composition comprises the combination together with one or more pharmaceutically acceptable carriers and/or excipients.

The invention also provides a combination as defined herein, prepared by a method as defined herein.

## BIOLOGICAL TEST METHODS

### PDE 3, PDE 4B, PDE 4D, PDE 5, PDE 6 Primary assay methods

The activity of the compounds can be measured in the assay methods shown below. Preferred compounds of the invention are selective PDE4 inhibitors, i.e. they inhibit PDE4 (e.g. PDE4B and/or PDE4D, preferably PDE4B) more strongly than they inhibit PDE3 and/or more strongly than they inhibit PDE5 and/or more strongly than they inhibit PDE6.

***PDE enzyme sources and literature references***

Human recombinant PDE4B, in particular the 2B splice variant thereof (HSPDE4B2B), is disclosed in WO 94/20079 and also M.M. McLaughlin et al., "A low *K*m, rolipram-sensitive, cAMP-specific phosphodiesterase from human brain: cloning and expression of cDNA, biochemical characterisation of recombinant protein, and tissue distribution of mRNA", *J. Biol. Chem.*, 1993, **268**, 6470-6476. For example, in Example 1 of WO 94/20079, human recombinant PDE4B is described as being expressed in the PDE-deficient yeast *Saccharomyces cerevisiae* strain GL62, e.g. after induction by addition of 150 uM $CuSO_4$, and 100,000 x *g* supernatant fractions of yeast cell lysates are described for use in the harvesting of PDE4B enzyme.

Human recombinant PDE4D (HSPDE4D3A) is disclosed in P. A. Baecker et al., "Isolation of a cDNA encoding a human rolipram-sensitive cyclic AMP phoshodiesterase (PDE $IV_D$)", *Gene*, 1994, **138**, 253-256.

Human recombinant PDE5 is disclosed in K. Loughney et al., "Isolation and characterisation of cDNAs encoding PDE5A, a human cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide phosphodiesterase", *Gene*, 1998, **216**, 139-147.

PDE3 was purified from bovine aorta as described by H. Coste and P. Grondin, "Characterisation of a novel potent and specific inhibitor of type V phosphodiesterase", *Biochem. Pharmacol.*, 1995, **50**, 1577-1585.

PDE6 was purified from bovine retina as described by: P. Catty and P. Deterre, "Activation and solubilization of the retinal cGMP-specific phosphodiesterase by limited proteolysis", *Eur. J. Biochem.*, 1991, **199**, 263-269; A. Tar et al. "Purification of bovine retinal cGMP phosphodiesterase", *Methods in Enzymology*, 1994, **238**, 3-12; and/or D. Srivastava et al. "Effects of magnesium on cyclic GMP hydrolysis by the bovine retinal rod cyclic GMP phosphodiesterase", *Biochem. J.*, 1995, **308**, 653-658.

***Inhibition of PDE 3, PDE 4B, PDE 4D, PDE 5 or PDE 6 activity: radioactive Scintillation Proximity Assay (SPA)***

The ability of compounds to inhibit catalytic activity at PDE4B or 4D (human recombinant), PDE3 (from bovine aorta), PDE5 (human recombinant) or PDE6 (from bovine retina) is determined by Scintillation Proximity Assay (SPA) in 96-well format. Test compounds (as a solution in DMSO, preferably about 2 microlitre (ul) volume of DMSO solution) are preincubated at ambient temperature (room temperature, e.g. 19-23°C) in Wallac Isoplates (code 1450-514) with PDE enzyme in 50mM Tris-HCl buffer pH 7.5 , 8.3mM $MgCl_2$, 1.7mM EGTA, 0.05% (w/v) bovine serum albumin for 10-30 minutes (usually 30 minutes). The enzyme concentration is adjusted so that no more than 20% hydrolysis of the substrate defined below occurred in control wells without compound, during the incubation. For the PDE3, PDE4B and PDE4D assays, [5',8-$^3$H]Adenosine 3',5'-cyclic phosphate (Amersham Pharmacia Biotech, code TRK.559; or Amersham Biosciences UK Ltd, Pollards Wood, Chalfont St Giles, Buckinghamshire HP8 4SP, UK) is added to give 0.05uCi per well and ~ 10nM final concentration. For the PDE5 and PDE6 assays, [8-$^3$H]Guanosine 3',5'-cyclic phosphate (Amersham Pharmacia Biotech, code TRK.392) is added to give 0.05uCi per well and ~ 36nM final concentration. Plates containing assay mixture, preferably approx. 100 ul volume of assay mixture, are mixed on an orbital shaker for 5 minutes and incubated at ambient temperature for 1 hour. Phosphodiesterase SPA beads (Amersham Pharmacia Biotech, code RPNQ 0150) are added (~1mg per well) to terminate the assay. Plates are sealed and shaken and allowed to stand at ambient temperature for 35 minutes to 1hour (preferably 35 minutes) to allow the beads to settle. Bound radioactive product is measured using a WALLAC TRILUX 1450 Microbeta scintillation counter. For inhibition curves, 10 concentrations (1.5nM - 30uM) of each compound are assayed. Curves are analysed using ActivityBase and XLfit (ID Business Solutions Limited, 2 Ocean Court, Surrey Research Park, Guildford, Surrey GU2 7QB, United Kingdom) Results are expressed as $pIC_{50}$ values.

In an alternative to the above radioactive SPA assay, PDE4B or PDE4D inhibition can be measured in the following Fluorescence Polarisation (FP) assay:

***Inhibition of PDE4B or PDE4D activity: Fluorescence Polarisation (FP) assay***

The ability of compounds to inhibit catalytic activity at PDE4B (human recombinant) or PDE4D (human recombinant) is determined by IMAP Fluorescence Polarisation (FP) assay (IMAP Explorer kit, available from Molecular Devices Corporation, Sunnydale, CA, USA; Molecular Devices code: R8062) in 384-well format. The IMAP FP assay is able to measure PDE activity in an homogenous, non-radioactive assay format. The FP assay uses the ability of immobilised trivalent metal cations, coated onto nanoparticles (tiny beads), to bind the phosphate group of Fl-AMP that is produced on the hydrolysis of fluorescein-labelled (Fl) cyclic adenosine mono-phosphate (Fl-cAMP) to the non-cyclic Fl-AMP form. Fl-cAMP does not bind. Binding of Fl-AMP product to the beads (coated with the immobilised trivalent cations) slows the rotation of the bound Fl-AMP and leads to an increase in the fluorescence polarisation ratio of parallel to perpendicular light. Inhibition of the PDE reduces/inhibits this signal increase.

Test compounds (small volume, e.g. ca. 0.5 to 1 ul, preferably ca. 0.5 ul, of solution in DMSO) are preincubated at ambient temperature (room temperature, e.g. 19-23°C) in black 384-well microtitre plates (supplier: NUNC, code 262260) with PDE enzyme in 10mM Tris-HCl buffer pH 7.2, 10mM $MgCl_2$, 0.1% (w/v) bovine serum albumin, and 0.05% $NaN_3$ for 10-30 minutes. The enzyme level is set by experimentation so that reaction was linear throughout the incubation. Fluorescein adenosine 3',5'-cyclic phosphate (from Molecular Devices Corporation, Molecular Devices code: R7091) is added to give about 40nM final concentration (final assay volume usually ca. 20-40 ul, preferably ca. 20 ul). Plates are mixed on an orbital shaker for 10 seconds and incubated at ambient temperature for 40 minutes. IMAP binding reagent (as described above, from Molecular Devices Corporation, Molecular Devices code: R7207) is added (60ul of a 1 in 400 dilution in binding buffer of the kit stock solution) to terminate the assay. Plates are allowed to stand at ambient temperature for 1 hour. The Fluorescence Polarisation (FP) ratio of parallel to perpendicular light is measured using an Analyst$^{TM}$ plate reader (from Molecular Devices Corporation). For inhibition curves, 10 concentrations (1.5nM - 30uM) of each compound are assayed. Curves are analysed using ActivityBase and XLfit (ID Business Solutions Limited, 2 Ocean Court, Surrey Research Park, Guildford, Surrey GU2 7QB, United Kingdom). Results are expressed as $pIC_{50}$ values.

In the FP assay, all reagents are dispensed using Multidrop$^{TM}$ (available from Thermo Labsystems Oy, Ratastie 2, PO Box 100, Vantaa 01620, Finland).

For a given PDE4 inhibitor, the PDE4B (or PDE4D) inhibition values measured using the SPA and FP assays can differ slightly. However, in a regression analysis of 100 test compounds (not necessarily compounds of the invention), the $pIC_{50}$ inhibition values measured using SPA and FP assays have been found generally to agree within 0.5 log units, for PDE4B and PDE4D (linear regression coefficient 0.966 for PDE4B and 0.971 for PDE4D; David R.Mobbs et al., "Comparison of the IMAP Fluorescence Polarisation Assay with the Scintillation Proximity Assay for Phosphodiesterase Activity", poster presented at 2003 Molecular Devices UK & Europe User Meeting, 2nd October 2003, Down Hall, Harlow, Essex, United Kingdom).

Biological Data obtained for some of the Examples (PDE4B inhibitory activity, either as one reading or as an average of ca. 2-6 readings) are as follows, based on current measurements only. In each of the SPA and FP assays, absolute accuracy of measurement is not possible, and the readings given are accurate only up to about ± 0.5 of a log unit, depending on the number of readings made and averaged:

| Example number | PDE4B $pIC_{50}$ (± about 0.5) |
|---|---|
| 1, 2 | 9.1 – 9-5 |
| 3, 4, 5, 8 | 8.1 – 9.0 |
| 6, 7, 9 | 6.1 – 7.0 |

*Emesis:* Some known PDE4 inhibitors can cause emesis and/or nausea to greater or lesser extents (e.g. see Z. Huang et al., *Current Opinion in Chemical Biology*, 2001, 5: 432-438, see especially pages 433-434 and refs cited therein). Therefore, it would be preferable, but not essential, if a PDE4 inhibitory compound or salt of the invention were to cause only limited or manageable emetic side-effects. Emetic side-effects can for example be measured by the emetogenic potential of the compound or salt when administered to ferrets; for example one can measure the time to onset, extent, frequency and/or duration of vomiting, retching and/or writhing in ferrets after oral or parenteral administration of the compound or salt. See for example In vivo Assay 4 hereinafter for a measurement method for anti-inflammatory effect, emetic side-effects and therapeutic index (TI) in the ferret. See also for example A. Robichaud et al., "Emesis induced by inhibitors of [PDE IV] in the ferret", *Neuropharmacology*, 1999, 38, 289-297, erratum *Neuropharmacology*, 2001, 40, 465-465. However, optionally, emetic side-effects and therapeutic index (TI) in rats can be conveniently measured by monitoring the pica feeding behaviour of rats after administration of the compound or salt of the invention (see In Vivo Assay 2 below).

*Other side effects:* Some known PDE4 inhibitors can cause other side effects such as headache and other central nervous sytem (CNS-) mediated side effects; and/or gastrointestinal (GI) tract disturbances. Therefore, it would be preferable but not essential if a particular PDE4 inhibitory compound or salt of the invention were to cause only limited or manageable side-effects in one or more of these side-effect categories.

**_In Vivo_ Biological Assays**

The *in vitro* enzymatic PDE4B inhibition assay described above should be regarded as being the primary test of biological activity. However, additional *in vivo* biological tests, which are optional and which are not an essential measure of efficacy or side-effects, are described below.

***In Vivo Assay 1. LPS-induced pulmonary neutrophilia in rats: effect of orally administered PDE4 inhibitors***

Pulmonary neutrophil influx has been shown to be a significant component to the family of pulmonary diseases like chronic obstructive pulmonary disease (COPD) which can involve chronic bronchitis and/or emphysema (G.F. Filley, *Chest.* 2000; 117(5);

251s-260s). The purpose of this neutrophilia model is to study the potentially anti-inflammatory effects *in vivo* of orally administered PDE4 inhibitors on neutrophilia induced by inhalation of aerosolized lipopolysaccharide (LPS), modelling the neutrophil inflammatory component(s) of COPD. See the literature section below for scientific background.

Male Lewis rats (Charles River, Raleigh, NC, USA) weighing approximately 300-400 grams are pretreated with either (a) test compound suspended in 0.5% methylcellulose (obtainable from Sigma-Aldrich, St Louis, MO, USA) in water or (b) vehicle only, delivered orally in a dose volume of 10 ml/kg. Generally, dose response curves are generated using the following doses of PDE4 inhibitors: 10.0, 2.0, 0.4, 0.08 and 0.016 mg/kg. Thirty minutes following pretreatment, the rats are exposed to aerosolized LPS (Serotype E. Coli 026:B6 prepared by trichloroacetic acid extraction, obtainable from Sigma-Aldrich, St Louis, MO, USA), generated from a nebulizer containing a 100 μg/ml LPS solution. Rats are exposed to the LPS aerosol at a rate of 4 L/min for 20 minutes. LPS exposure is carried out in a closed chamber with internal dimensions of 45 cm length x 24 cm width x 20 cm height. The nebulizer and exposure chamber are contained in a certified fume hood. At 4 hours-post LPS exposure the rats are euthanized by overdose with pentobarbital at 90 mg/kg, administered intraperitoneally. Bronchoalveolar lavage (BAL) is performed through a 14 gauge blunt needle into the exposed trachea. Five, 5 ml washes are performed to collect a total of 25 ml of BAL fluid. Total cell counts and leukocyte differentials are performed on BAL fluid in order to calculate neutrophil influx into the lung. Percent neutrophil inhibition at each dose (cf. vehicle) is calculated and a variable slope, sigmoidal dose-response curve is generated, usually using Prism Graph-Pad. The dose-response curve is used to calculate an ED50 value (in mg per kg of body weight) for inhibition by the PDE4 inhibitor of the LPS-induced neutrophilia.

*Alternative method :* In an alternative embodiment of the procedure, a single oral dose of 10 mg/kg or 1.0 mg/kg of the PDE4 inhibitor (or vehicle) is administered to the rats, and percent neutrophil inhibition is calculated and reported for that specific dose.

*Literature:*

Filley G.F. Comparison of the structural and inflammatory features of COPD and asthma. *Chest.* 2000; 117(5) 251s-260s.

Howell RE, Jenkins LP, Fielding LE, and Grimes D. Inhibition of antigen-induced pulmonary eosinophilia and neutrophilia by selective inhibitors of phosphodiesterase types 3 and 4 in brown Norway rats. *Pulmonary Pharmacology.* 1995; 8: 83-89.

Spond J, Chapman R, Fine J, Jones H, Kreutner W, Kung TT, Minnicozzi M. Comparison of PDE 4 inhibitors, Rolipram and SB 207499 (Ariflo™), in a rat model of pulmonary neutrophilia. *Pulmonary Pharmacology and Therapeutics.* 2001; 14: 157-164.

Underwood DC, Osborn RR, Bochnowicz S, Webb EF, Rieman DJ, Lee JC, Romanic AM, Adams JL, Hay DWP, and Griswold DE. SB 239063, a p38 MAPK inhibitor, reduces neutrophilia, inflammatory cytokines, MMP-9, and fibrosis in lung. *Am J Physiol Lung Cell Mol Physiol.* 2000; 279: L895-L902.

***In Vivo Assay 2.*** ***Rat Pica Model of emesis***

*Background:* Selective PDE4 inhibitors have been shown to inhibit inflammation in various *in vitro* and *in vivo* models by increasing intracellular levels of cAMP of many immune cells (e.g. lymphocytes, monocytes). However, a side effect of some PDE4 inhibitors in many species is emesis. Because many rat models of inflammation are well characterized, they have been used in procedures (see e.g. In Vivo Assay 1 above) to show beneficial anti-inflammatory effects of PDE 4 inhibitors. However rats have no emetic response (they have no vomit reflex), so that the relationship between beneficial anti-inflammatory effects of PDE 4 inhibitors and emesis is difficult to study directly in rats.

However, in 1991, Takeda *et al.* (see Literature section below) demonstrated that the pica feeding response is analogous to emesis in rats. Pica feeding is a behavioural response to illness in rats wherein rats eat non-nutritive substances such as earth or in particular clay (e.g. kaolin) which may help to absorb toxins. Pica feeding can be induced by motion and chemicals (especially chemicals which are emetic in humans), and can be inhibited pharmacologically with drugs that inhibit emesis in humans. The Rat Pica Model, In Vivo Assay 2, can determine the level of pica response of rats to PDE 4 inhibition at pharmacologically relevant doses in parallel to *in vivo* anti-inflammatory Assays in (a separate set of) rats (e.g. In Vivo Assay 1 above).

Anti-inflammatory and pica assays in the same species together can provide data on the "therapeutic index" (TI) in the rat of the compounds/salts of the invention. The Rat TI can for example be calculated as the ratio of a) the potentially-emetic Pica Response ED50 dose from Assay 2 to b) the rat anti-inflammatory ED50 dose (e.g. measured by rat neutrophilia-inhibition in eg In Vivo Assay 1), with larger TI ratios possibly indicating lower emesis at many anti-inflammatory doses. This might allow a choice of a non-emetic or minimal-emetic pharmaceutical dose of the compounds or salts of the invention which has an anti-inflammatory effect. It is recognised however that achieving a low-emetic PDE4 inhibitory compound is not essential to the invention.

*Procedure:* On the first day of the experiment, the rats are housed individually in cages without bedding or "enrichment". The rats are kept off of the cage floor by a wire screen. Pre-weighed food cups containing standard rat chow and clay pellets are placed in the cage. The clay pellets, obtainable from Languna Clay Co, City of Industry, CA, USA, are the same size and shape as the food pellets. The rats are acclimated to the clay for 72 hours, during which time the cups and food and clay debris from the cage are weighed daily on an electronic balance capable of measuring to the nearest 0.1 grams. By the end of the 72 hour acclimation period the rats generally show no interest in the clay pellets.

At the end of 72 hours the rats are placed in clean cages and the food cups weighed. Rats that are still consuming clay regularly are removed from the study. Immediately prior to the dark cycle (the time when the animals are active and should be eating) the animals are split into treatment groups and dosed orally with a dose of the compound/salt of the invention (different doses for different treatment groups) or with vehicle alone, at a dose volume of 2 ml/kg. In this oral dosing, the compound/salt is in the form of a suspension in 0.5% methylcellulose (obtainable Sigma-Aldrich, St. Louis, MO, USA) in water. The food and clay cups and cage debris are weighed the following day and the total clay and food consumed that night by each individual animal is calculated.

A dose response is calculated by first converting the data into quantal response, where animals are either positive or negative for the pica response. A rat is "pica

positive" if it consumes greater than or equal to 0.3 grams of clay over the mean of is usually calculated using logistic regression performed by the Statistica software statistical package. A Pica Response ED50 value in mg per kg of body weight can then be calculated.

The Pica Response ED50 value can be compared to the neutrophilia-inhibition ED50 values for the same compound administered orally to the rat (measurable by In Vivo Assay 1 above), so that a Therapeutic Index (TI) in rats can be calculated thus:

$$\text{Rat Therapeutic index (TI) (50/50)} = \frac{\text{Pica Response ED50 value}}{\text{rat neutrophilia-inhibition ED50 value}}$$

In general, the Therapeutic Index (TI) calculated this way is often substantially different to, and for example can often be substantially higher than, the TI (D20/D50) calculated in the ferret (see In vivo Assay 4 below).

*Literature:*

Beavo JA, Contini, M., Heaslip, R.J. Multiple cyclic nucleotide phosphodiesterases. *Mol Pharmacol.* 1994; 46:399-405.

Spond J, Chapman R, Fine J, Jones H, Kreutner W, Kung TT, Minnicozzi M. Comparison of PDE 4 inhibitors, Rolipram and SB 207499 (Ariflo™), in a rat model of pulmonary neutrophilia. *Pulmonary Pharmacology and Therapeudtics*. 2001; 14:157-164.

Takeda N, Hasegawa S, Morita M, and Matsunaga T. Pica in rats is analogous to emesis: an animal model in emesis research. *Pharmacology, Biochemistry and Behavior.* 1991; 45:817-821.

Takeda N, Hasegawa S, Morita M, Horii A, Uno A, Yamatodani A and Matsunaga T. Neuropharmacological mechanisms of emesis. I . Effects of antiemetic drugs on motion- and apomorphine-induced pica in rats. *Meth Find Exp Clin Pharmacol.* 1995; 17(9) 589-596.

Takeda N, Hasegawa S, Morita M, Horii A, Uno A, Yamatodani A and Matsunaga T. Neuropharmacological mechanisms of emesis. II . Effects of antiemetic drugs on cisplatin-induced pica in rats. *Meth Find Exp Clin Pharmacol.* 1995; 17(9) 647-652.

***In Vivo Assay 3. LPS induced pulmonary neutrophilia in rats: effect of intratracheally administered PDE4 inhibitors***

This assay is an animal model of inflammation in the lung – specifically neutrophilia induced by lipopolysaccharide (LPS) – and allows the study of putative inhibition of such neutrophilia (anti-inflammatory effect) by intratracheally (i.t.) administered PDE4 inhibitors. The PDE4 inhibitors are preferably in dry powder or wet suspension form. I.t. administration is one model of inhaled administration, allowing topical delivery to the lung.

*Animals:* Male CD (Sprague Dawley Derived) rats supplied by Charles River, Raleigh, NC, USA are housed in groups of 5 rats per cage, acclimatised after delivery for at least 7 days with bedding/nesting material regularly changed, fed on SDS diet R1

pelleted food given *ad lib,* and supplied with daily-changed pasteurised animal grade drinking water.

*Device for dry powder administration:* Disposable 3-way tap between dosing needle and syringe. A 3-way sterile tap (Vycon Ref 876.00) is weighed, the drug blend or inhalation grade lactose (vehicle control) is then added to the tap, the tap closed to prevent loss of drug, and the tap is re-weighed to determine the weight of drug in the tap. After dosing, the tap is weighed again to determine the weight of drug that had left the tap. The needle, a Sigma Z21934-7 syringe needle 19-gauge 152 mm (6 inches) long with luer hub, is cut by engineering to approximately 132 mm (5.2 inches), a blunt end is made to prevent them damaging the rat's trachea, and the needle is weighed prior to and after drug delivery to confirm that no drug was retained in the needles after dosing.

*Device for wet suspension administration:* This is the similar to the above but a blunt dosing needle, whose forward end was slightly angled to the needle axis, is used, with a flexible plastic portex canula inserted into the needle.

*Drugs and Materials:* Lipopolysaccharide (LPS) (Serotype:0127:B8) (L3129 Lot 61K4075) is dissolved in phosphate-buffered saline (PBS). PDE4 inhibitors are used in size-reduced (e.g. micronised) form, for example according to the Micronisation Example given above. For dry powder administration of the drug, the Dry Powder Formulation Example given above, comprising drug and inhalation-grade lactose, can be used. The inhalation-grade lactose usually used (Lot E98L4675 Batch 845120) has 10% fines (10% of material under 15um particle size measured by Malvern particle size).

Wet suspensions of the drug can be prepared by adding the required volume of vehicle to the drug; the vehicle used being a mixture of saline/tween (0.2% tween 80). The wet suspension is sonicated for 10 minutes prior to use.

*Preparation, and dosing with PDE 4 inhibitor:* Rats are anaesthetised by placing the animals in a sealed Perspex chamber and exposing them to a gaseous mixture of isoflourane (4.5 %), nitrous oxide (3 litres.minute$^{-1}$) and oxygen (1 litre.minute$^{-1}$). Once anaesthetised, the animals are placed onto a stainless steel i.t. dosing support table. They are positioned on their back at approximately a 35° angle. A light is angled against the outside of the throat to highlight the trachea. The mouth is opened and the opening of the upper airway visualised. The procedure varies for wet suspension and dry powder administration of PDE4 inhibitors as follows:

*Dosing with a Wet suspension:* A portex cannula is introduced via a blunt metal dosing needle that has been carefully inserted into the rat trachea. The animals are intratracheally dosed with vehicle or PDE4 inhibitor via the dosing needle with a new internal canula used for each different drug group. The formulation is slowly (10 seconds) dosed into the trachea using a syringe attached to the dosing needle.

*Dosing with a Dry Powder:* The three-way tap device and needle are inserted into the rat trachea up to a pre-determined point established to be located approximately 1 cm above the primary bifurcation. Another operator holds the needle at the specified position whilst 2x 4ml of air is delivered through the three-way tap by depressing the syringes (ideally coinciding with the animal inspiring), aiming to expel the entire drug quantity

from the tap. After dosing, the needle and tap are removed from the airway and the tap closed off to prevent any retained drug leaving the tap.

After dosing with either wet suspension or dry powder, the animals are then removed from the table and observed constantly until they have recovered from the effects of anaesthesia. The animals are returned to the holding cages and given free access to food and water; they are observed and any unusual behavioural changes noted.

*Exposure to LPS:* About 2 hours after i.t. dosing with vehicle control or the PDE4 inhibitor, the rats are placed into sealed Perspex containers and exposed to an aerosol of LPS (nebuliser concentration 150 $\mu g.ml^{-1}$) for 15 minutes. Aerosols of LPS are generated by a nebuliser (DeVilbiss, USA) and this is directed into the Perspex exposure chamber. Following the 15-minute LPS-exposure period, the animals are returned to the holding cages and allowed free access to both food and water.

[In an alternative embodiment, the rats can exposed to LPS less than 2 hours after i.t. dosing. In another alternative embodiment, the rats can exposed to LPS more than 2 hours (e.g. ca. 4 or ca. 6 hours) after i.t. dosing by vehicle or PDE4 inhibitor, to test whether or not the PDE4 inhibitor has a long duration of action (which is not essential).]

*Bronchoalveolar lavage:* 4 hours after LPS exposure the animals are killed by overdose of sodium pentobarbitone (i.p.). The trachea is cannulated with polypropylene tubing and the lungs are lavaged (washed out) with 3 x 5 mls of heparinised (25 $units.ml^{-1}$) phosphate buffered saline (PBS).

*Neutrophil cell counts:* The Bronchoalveolar lavage (BAL) samples are centrifuged at 1300 rpm for 7 minutes. The supernatant is removed and the resulting cell pellet resuspended in 1 ml PBS. A cell slide of the resuspension fluid is prepared by placing 100µl of resuspended BAL fluid into cytospin holders and then is spun at 5000 rpm for 5 minutes. The slides are allowed to air dry and then stained with Leishmans stain (20 minutes) to allow differential cell counting. The total cells are also counted from the resuspension. From these two counts, the total numbers of neutrophils in the BAL are determined. For a measure of PDE4-inhibitor-induced inhibition of neutrophilia, a comparison of the neutrophil count in rats treated with vehicle and rats treated with PDE4 inhibitors is conducted.

By varying the dose of the PDE4 inhibitor used in the dosing step (e.g. 0.2 or 0.1 mg of PDE4 inhibitor per kg of body weight, down to e.g. 0.01 mg/kg), a dose-response curve can be generated.

***In Vivo Assay 4. Evaluation of Therapeutic Index of Orally-administered PDE 4 inhibitors in the conscious ferret***

*1.1 Materials*

The following materials are used for these studies:

PDE4 inhibitors are prepared for oral (p.o.) administration by dissolving in a fixed volume (1 ml) of acetone and then adding cremophor to 20% of the final volume. Acetone is evaporated by directing a flow of nitrogen gas onto the solution. Once the acetone is removed, the solution is made up to final volume with distilled water. LPS is dissolved in phosphate buffered saline.

*1.2 Animals*

Male ferrets (Mustela Pulorius Furo, weighing 1 – 2 kg) are transported and allowed to acclimatise for not less than 7 days. The diet comprises SDS diet C pelleted food given *ad lib* with Whiskers$^{TM}$ cat food given 3 times per week. The animals are supplied with pasteurised animal grade drinking water changed daily.

*1.3 Experimental Protocol(s)*

*1.3.1 Dosing with PDE4 inhibitors*

PDE4 inhibitors are administered orally (p.o.), using a dose volume of 1ml/kg. Ferrets are fasted overnight but allowed free access to water. The animals are orally dosed with vehicle or PDE 4 inhibitor using a 15cm dosing needle that is passed down the back of the throat into the oesophagus. After dosing, the animals are returned to holding cages fitted with perspex doors to allow observation, and given free access to water. The animals are constantly observed and any emetic episodes (retching and vomiting) or behavioural changes are recorded. The animals are allowed access to food 60 – 90 minutes after p.o. dosing.

*1.3.2 Exposure to LPS*

Thirty minutes after oral dosing with compound or vehicle control, the ferrets are placed into sealed perspex containers and exposed to an aerosol of LPS (30 μg/ml) for 10 minutes. Aerosols of LPS are generated by a nebuliser (DeVilbiss, USA) and this is directed into the perspex exposure chamber. Following a 10-minute exposure period, the animals are returned to the holding cages and allowed free access to water, and at a later stage, food. General observation of the animals continues for a period of at least 2.5 hours post oral dosing. All emetic episodes and behavioural changes are recorded.

*1.3.3 Bronchoalveolar lavage and cell counts*

Six hours after LPS exposure the animals are killed by overdose of sodium pentobarbitone administered intraperitoneally. The trachea is then cannulated with polypropylene tubing and the lungs lavaged twice with 20 ml heparinised (10 units/ml) phosphate buffered saline (PBS). The bronchoalveolar lavage (BAL) samples are centrifuged at 1300 rpm for 7 minutes. The supernatant is removed and the resulting cell pellet re-suspended in 1 ml PBS. A cell smear of re-suspended fluid is prepared and stained with Leishmans stain to allow differential cell counting. A total cell count is made using the remaining re-suspended sample. From this, the total number of neutrophils in the BAL sample is determined.

*1.3.4 Pharmacodynamic readouts*

The following parameters are recorded:
a) % inhibition of LPS-induced pulmonary neutrophilia to determine the dose of PDE4 inhibitor which gives 50% inhibition (D50).
b) Emetic episodes – the number of vomits and retches are counted to determine the dose of PDE4 inhibitor that gives a 20% incidence of emesis (D20).
c) A therapeutic index (TI), using this assay, is then calculated for each PDE4 inhibitor using the following equation:

$$\text{Ferret Therapeutic index (TI) (D20/D50)} = \frac{\text{D20 incidence of emesis in ferret}}{\text{D50 inhibition of neutrophilia in ferret}}$$

It is noted that the Ferret Therapeutic index (TI) (D20/D50) calculated using this in vivo Assay 4 is often substantially different to, and for example is often substantially lower than, the Rat TI (50/50) calculated using the rat oral inflammation and pica feeding Assays 1+2.

The calculation of Ferret TI using the known PDE4 inhibitor roflumilast in this Assay 4 is:
D20 for emesis = about 0.46 mg/kg p.o.,
D50 for ferret neutroplilia = about 0.42 mg/kg p.o.,
**Ferret TI = about 1.1.**

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

## EXAMPLES

The various aspects of the invention will now be described by reference to the following examples. These examples are merely illustrative and are not to be construed as a limitation of the scope of the present invention. In this section, "Intermediates" represent syntheses of intermediate compounds intended for use in the synthesis of the "Examples".

**Abbreviations** used herein:

| | |
|---|---|
| DCM | dichloromethane |
| DMF | dimethyl formamide |
| DIPEA | diisopropylethyl amine ($^{i}Pr_2NEt$) |
| EDC | 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride |
| EtOAc | ethyl acetate |
| EtOH | ethanol |
| HATU | O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate |
| HCl | hydrogen chloride or hydrochloric acid |
| HOBT | hydroxybenzotriazole = 1-hydroxybenzotriazole |
| MeCN | acetonitrile |
| MeOH | methanol |
| $NaHCO_3$ | sodium bicarbonate |
| NaOH | sodium hydroxide |
| $Na_2SO_4$ | sodium sulfate |
| KOH | potassium hydroxide |
| THF | tetrahydrofuran |
| | |
| HPLC | high pressure liquid chromatography |
| SPE | solid phase extraction |
| NMR | nuclear magnetic resonance (in which: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet, H = no. of protons) |
| LCMS | liquid chromatography/mass spectroscopy |
| TLC | thin layer chromatography |
| h | hours |
| $T_{RET}$ | retention time |
| Room temperature | this is usually in the range of about 20 to about 25 °C. |

**General Experimental Details**

**Machine Methods** used herein:

*LCMS (liquid chromatography/mass spectroscopy)*

Waters ZQ mass spectrometer operating in positive ion electrospray mode, mass range 100-1000 amu.
UV wavelength : 215-330nM
Column : 3.3cm x 4.6mm ID, 3µm ABZ+PLUS
Flow Rate : 3ml/min
Injection Volume : 5µl
Solvent A : 95% acetonitrile + 0.05% formic acid
Solvent B : 0.1% formic acid + 10mMolar ammonium acetate
Gradient : 0% A/0.7min, 0-100% A/3.5min, 100% A/1.1min, 100-0% A/0.2min
It should be noted that retention times ($T_{RET}$) quoted herein may vary slightly (+/- 0.1min.) when samples were run on different Waters machines, even though the same type of column and identical flow rates, injection volumes, solvents and gradients were used.

*Mass directed autoprep HPLC*

The prep column used was a Supelcosil ABZplus (10cm x 2.12cm) (usually 10cm x 2.12cm x 5 µm).
UV wavelength : 200-320nM
Flow : 20ml/min
Injection Volume: 1ml; or more preferably 0.5 ml
Solvent A : 0.1% formic acid
Solvent B : 95% acetonitrile + 5% formic acid; or more usually 99.95% acetonitrile + 0.05% formic acid
Gradient : 100% A/1min, 100-80% A/9min, 80-1% A/3.5min, 1% A/1.4min, 1-100%A/0.1min

**Chiral Columns for Chromatographic Purification**

ChiralPak AS columns were obtained from:
Chiral Technologies Europe Sarl, Illkirch, France (Telephone: 0033(0)388795200; (cte@chiral.fr; www.chiral.fr).

**Intermediates and Examples**

All reagents not detailed in the text below are commercially available from established suppliers such as Sigma-Aldrich. The addresses of the suppliers for some of the starting materials mentioned in the Intermediates and Examples below or the Assays above are as follows:

- Aldrich (catalogue name), Sigma-Aldrich Company Ltd., Dorset, United Kingdom, telephone: +44 1202 733114; Fax: +44 1202 715460; ukcustsv@eurnotes.sial.com; or
- Aldrich (catalogue name), Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178-9916, USA; telephone: 314-771-5765; fax: 314-771-5757; custserv@sial.com; or

- Aldrich (catalogue name), Sigma-Aldrich Chemie Gmbh, Munich, Germany; telephone: +49 89 6513 0; Fax: +49 89 6513 1169; deorders@eurnotes.sial.com.
- Fluka Chemie AG, Industriestrasse 25, P.O. Box 260, CH-9471 Buchs, Switzerland
- Lancaster Synthesis Ltd., Newgate, White Lund, Morecambe, Lancashire LA3 3DY, United Kingdom

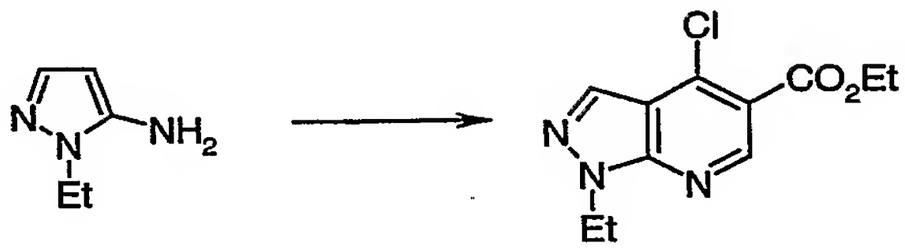
Trans World Chemicals, Inc., 14674 Southlawn Lane, Rockville, MD 20850, USA

**Table of Intermediates**

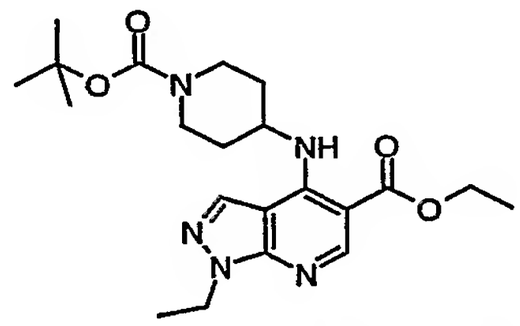
| Int No | Name |
|---|---|
| **1** | Ethyl 4-chloro-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate |
| **2** | Ethyl 4-[(1-{[(1,1-dimethylethyl)oxy]carbonyl}-4-piperidinyl)amino]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate |
| **3** | Ethyl 1-ethyl-4-(4-piperidinylamino)-1H-pyrazolo[3,4-b]pyridine-5-carboxylate hydrochloride |
| **4** | Ethyl 4-{[1-(aminocarbonyl)-4-piperidinyl]amino}-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate |
| **5** | 4-{[1-(aminocarbonyl)-4-piperidinyl]amino}-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid |
| **6** | 4-chloro-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid |
| **7** | 4-chloro-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carbonyl chloride |
| **8** | 4-chloro-N-[(2,4-dimethylphenyl)methyl]-1-ethyl-1H-pyrazolo[3,4-b] pyridine-5-carboxamide |
| **9** | 4-chloro-N-[(3,4-dimethylphenyl)methyl]-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide |
| **10** | 4-chloro-1-ethyl-N-{[4-(methyloxy)phenyl]methyl}-1H-pyrazolo[3,4-b]pyridine-5-carboxamide |
| **11** | 1,1-dimethylethyl [1-(aminocarbonyl)-4-piperidinyl]carbamate |
| **12** | 4-amino-1-piperidinecarboxamide hydrochloride |
| **13** | 1,1-dimethylethyl [4-(aminocarbonyl)cyclohexyl]carbamate |
| **14** | : 1,1-dimethylethyl {4-[(methylamino)carbonyl]cyclohexyl}carbamate |
| **15** | 4-aminocyclohexanecarboxamide hydrochloride |
| **16** | 4-amino-*N*-methylcyclohexanecarboxamide |

**Intermediate 1: Ethyl 4-chloro-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate**

Prepared from commercially available 5-amino-1-ethyl pyrazole as described by G. Yu et. al. in *J. Med Chem.*, 2001, 44, 1025-1027:

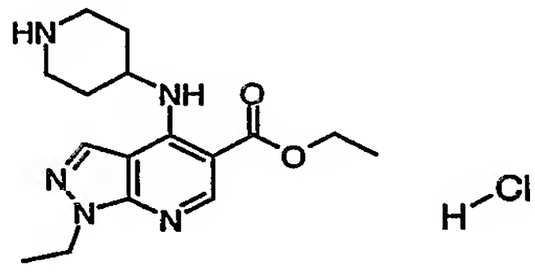


**<u>Intermediate 2</u>: Ethyl 4-[(1-{[(1,1-dimethylethyl)oxy]carbonyl}-4-piperidinyl)amino]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate**



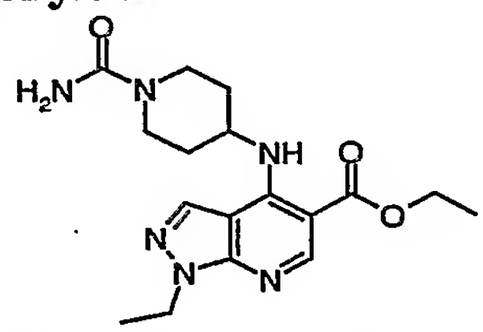
A solution of Intermediate 1 (2.3g) in MeCN (50ml) was treated with solid 1,1-dimethylethyl 4-amino-1-piperidinecarboxylate (2g) and DIPEA (8.6ml). The reaction mixture was heated at 90°C for 16h. The solvents were removed under reduced pressure and the residue was partitioned between DCM (100ml) and water (75ml). The organic fraction was collected through a hydrophobic frit and the solvents were removed under reduced pressure to yield Intermediate 2 as a white solid (3.9g). LCMS showed $MH^+$ = 418; $T_{RET}$ = 3.35min.

**<u>Intermediate 3</u>: Ethyl 1-ethyl-4-(4-piperidinylamino)-1H-pyrazolo[3,4-b]pyridine-5-carboxylate hydrochloride**



Intermediate 2 (3.9g) was treated with 4.0M HCl in 1, 4-dioxane (30ml) and the reaction mixture was stirred at 22°C for 1h. The solvents were removed to give Intermediate 3 as a white solid (3.9g). LCMS showed $MH^+$ = 318; $T_{RET}$ = 2.21min.

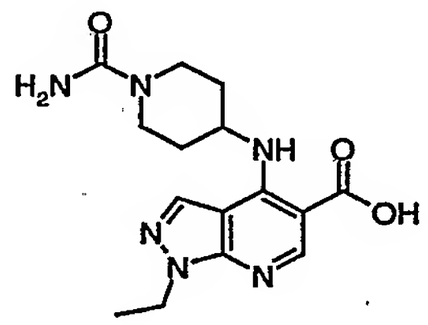
**<u>Intermediate 4</u>: Ethyl 4-{[1-(aminocarbonyl)-4-piperidinyl]amino}-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate**



A suspension of intermediate 3 (3.9g) in THF (100ml) was treated with trimethylsilyl isocyanate (1.99ml) followed by DIPEA (2.6ml) and the solution was stirred at 22°C for 2h. The volatile solvents were removed under reduced pressure and the residue was

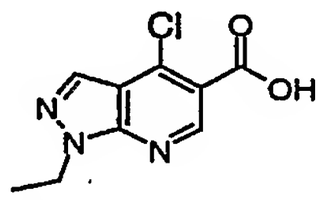
partitioned between DCM (50ml) and water (25ml). The organic layer was collected. The aqueous phase was re-extracted with DCM (50ml). The organic layers were combined, separated from water by passing through a hydrophobic frit and concentrated under reduced pressure to yield Intermediate 4 as a white solid (3.9g). LCMS showed $MH^+$ = 361; $T_{RET}$ = 2.45min.

**<u>Intermediate 5:</u> 4-{[1-(aminocarbonyl)-4-piperidinyl]amino}-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid**



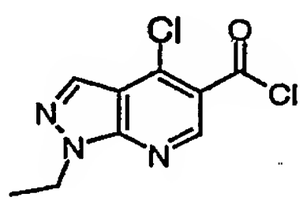
A solution of intermediate 4 (3.9g) in EtOH (50ml) was treated with a solution of NaOH (1.77g) in water (20ml) and the reaction mixture was heated at 80°C for 16h. LCMS indicated that partial hydrolysis of the urea portion had occurred. The solvents were removed and the residue was dissolved in water (5ml), the pH was adjusted to 3 (2M HCl) and the resultant white precipitate was collected by filtration and dried. This precipitate was dissolved inEtOH. The solution was treated with trimethylsilyl isocyanate (3ml) and DIPEA (10ml) and then stirred at 22°C for 16h. The solvents were removed and the residue was dissolved in water (5ml), the pH was adjusted to 3 (2M HCl) and the resultant white precipitate was collected by filtration and dried to give Intermediate 5 as a white solid (2.66g). LCMS showed $MH^+$ = 333; $T_{RET}$ = 2.00min.

**<u>Intermediate 6:</u> 4-chloro-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid**



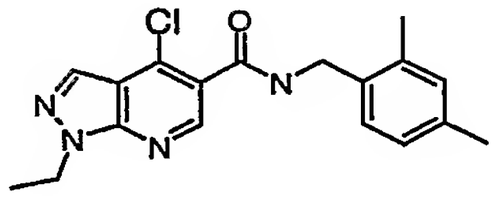
A solution of intermediate 1 (20g) in 1,4-dioxane (100ml) was treated with a solution of KOH (18g) in water (30ml) and the reaction mixture was stirred at 22°C for 24h. The solvent was evaporated and the residue was acidified to pH3 (2M HCl). The resultant white precipitate was collected by filtration and dried to give Intermediate 6 as a white solid (16.9g). LCMS showed $MH^+$ = 226; $T_{RET}$ = 2.45min.

**<u>Intermediate 7:</u> 4-chloro-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carbonyl chloride**



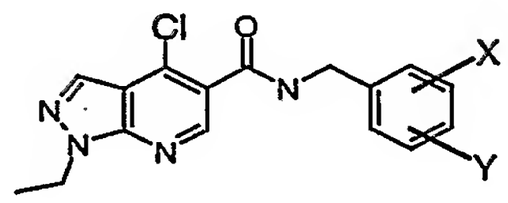
A solution of intermediate 106 (17.8g) in thionyl chloride (100ml) was heated under reflux for 3.5h. The solution was cooled to room temperature. The thionyl chloride was removed *in vacuo* and any remaining thionyl chloride was removed by azeotropic distillation with toluene (30ml) to give Intermediate 7 as a beige solid (16.8g). LCMS (MeOH solution) showed $MH^+ = 240$ (Methyl ester); $T_{RET} = 2.88$min.

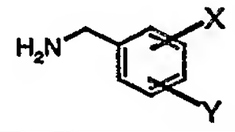
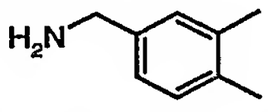
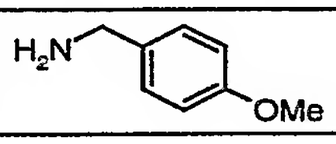
**<u>Intermediate 8</u>: 4-chloro-N-[(2,4-dimethylphenyl)methyl]-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide**



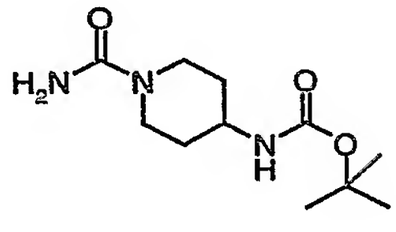
A solution of Intermediate 7 (8.2mmol) in THF (20ml) was treated with 2,4-dimethyl benzylamine (Trans World Chemicals; 8.2mmol) and DIPEA (8.2mmol). The reaction mixture was stirred at 22°C for 24h. The solvents were evaporated and the residue was dissolved in DCM (50ml). The solution was washed with 5% citric acid solution (50ml) and 0.5M $NaHCO_3$ solution (50ml), dried ($Na_2SO_4$), filtered and evaporated to give Intermediate 8 as a white solid (1.61g). LCMS showed $MH^+ = 343$; $T_{RET} = 3.22$min.

The following Intermediates 9 and 10 were prepared in a similar manner from Intermediate 7 and the appropriate amine reagent:



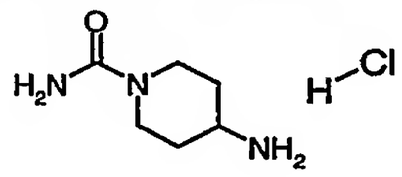
| | Amine reagent: | Source of amine reagent | $MH^+$ ion | $T_{RET}$ (min) |
|---|---|---|---|---|
| |  | | | |
| **Intermediate 9** |  | Trans World Chemicals | 343 | 3.34 |
| **Intermediate 10** |  | Aldrich | 345 | 2.9 |

**<u>Intermediate 11:</u> 1,1-dimethylethyl [1-(aminocarbonyl)-4-piperidinyl]carbamate**



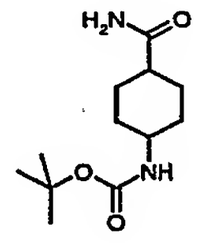
A solution of 1,1-dimethylethyl 4-piperidinylcarbamate (0.35g) in DCM (10ml) was treated with trimethylsilyl isocyanate (1.1ml). The reaction mixture was stirred at 22°C for 72h. The mixture was diluted with saturated $NaHCO_3$ (20ml). The organic phase was collected through a hydrophobic frit and evaporated to give Intermediate 11 as a white foam (0.29g). $^1H$ NMR (400MHz in $CDCl_3$, 27°C, δ ppm) 4.45 (br. s, 3H). 3.90 (d, 2H), 3.65 (br. m, 1H), 2.9-3.0 (dt, 2H), 1.95-2.0 (br. dd, 2H), 1.45 (s, 9H), 1.3-1.4 (dq, 2H).

**<u>Intermediate 12:</u> 4-amino-1-piperidinecarboxamide hydrochloride**




A solution of intermediate 11 (0.29g) in 4.0M HCl in 1, 4-dioxane (5ml) was stirred at 22°C for 4h. The solvent was evaporated to give Intermediate 12 as a white foam (0.27g). $^1H$ NMR (400MHz in $d_6$-DMSO, 27°C, δ ppm) 8.1 (br. s, 2H), 3.95 (d, 2H), 3.15 (m, 1H), 2.7 (dt, 2H), 1.85 (dd, 2H), 1.35 (m, 2H).

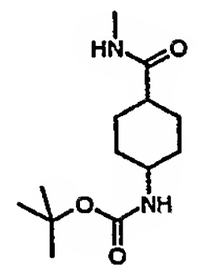
**<u>Intermediate 13:</u> 1,1-dimethylethyl [4-(aminocarbonyl)cyclohexyl]carbamate**




A solution of 4-({[(1,1-dimethylethyl)oxy]carbonyl}amino)cyclohexanecarboxylic acid (ex. Fluka, 1g) in DMF (30ml) was treated with HATU (1.72g) and DIPEA (5.4ml). The reaction mixture was stirred at 22°C for 10 min. A 0.5M solution of ammonia in 1,4-dioxane (40ml) was added and the reaction mixture was stirred at 22°C for 72h. The solvents were evaporated and the residue was purified by loading the crude mixture onto a 50g aminopropyl SPE cartridge and eluting with EtOAc (100ml), then MeOH (100ml). Intermediate 13 was isolated by evaporation of the MeOH fraction as a yellow oil (0.99g). LCMS showed $MH^+ = 242$; $T_{RET} = 2.2$min.

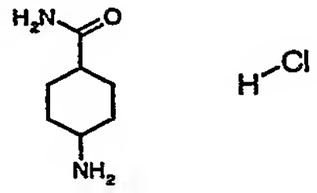
The following Intermediate 13 was prepared in a similar manner from 4-({[(1,1-dimethylethyl)oxy]carbonyl}amino)cyclohexanecarboxylic acid and methylamine:

**<u>Intermediate 14:</u> 1,1-dimethylethyl {4-[(methylamino)carbonyl]cyclohexyl}carbamate**



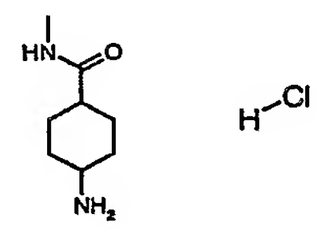
LCMS showed $MH^+ = 256$; $T_{RET} = 2.4$min.

**Intermediate 15: 4-aminocyclohexanecarboxamide hydrochloride**



4.0M HCl in 1;4-dioxane (14ml) was added to intermediate 13 (0.99g) and the reaction mixture was stirred at 22°C for 30min. The solvent was evaporated to give Intermediate 15 as a yellow gum (1.03g). $^1$H NMR (400MHz in $d_6$-DMSO, 27°C, δppm) 7.9 (br. S, 2H), 3.9 (br. S, 2H), 3.10 (m, 1H), 1.92 (m, 2H), 1.68 (m, 4H), 1.50 (m, 2H).

The following Intermediate 16 was prepared in a similar manner from Intermediate 14:



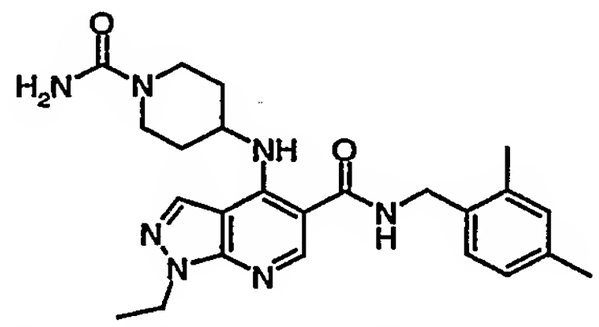
$^1$H NMR (400MHz in $d_6$-DMSO, 27°C, δppm) 8.1 (br. S, 1H), 6.5 (br. S, 2H), 3.2 (m, 1H), 2.49 (s, 3H), 1.98 (m, 2H), 1.61 (m, 4H), 1.50 (m, 2H).

**Table of Examples**

| Example Number | Name |
|---|---|
| 1 | 4-{[1-(aminocarbonyl)-4-piperidinyl]amino}-N-[(2,4-dimethylphenyl)methyl]-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide |

| | |
|---|---|
| **2** | **4-{[1-(aminocarbonyl)-4-piperidinyl]amino}-N-[(3,4-dimethylphenyl)methyl]-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide** |
| **3** | **4-{[1-(aminocarbonyl)-4-piperidinyl]amino}-1-ethyl-N-{[4-(methyloxy)phenyl]methyl}-1H-pyrazolo[3,4-b]pyridine-5-carboxamide** |
| **4** | **4-{[4-(aminocarbonyl)cyclohexyl]amino}-*N*-[(3,4-dimethylphenyl)methyl]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide** |
| **5** | **4-{[4-(aminocarbonyl)cyclohexyl]amino}-*N*-[(2,4-dimethylphenyl)methyl]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide** |
| **6** | ***N*-[(2,4-dimethylphenyl)methyl]-1-ethyl-4-({4-[(methylamino)carbonyl]cyclohexyl}amino)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide** |
| **7** | ***N*-[(3,4-dimethylphenyl)methyl]-1-ethyl-4-({4-[(methylamino)carbonyl]cyclohexyl}amino)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide** |
| **8** | **(cis)-4-{[4-(aminocarbonyl)cyclohexyl]amino}-*N*-[(3,4-dimethylphenyl)methyl]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide** |
| **9** | **(trans)-4-{[4-(aminocarbonyl)cyclohexyl]amino}-*N*-[(3,4-dimethylphenyl)methyl]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide** |

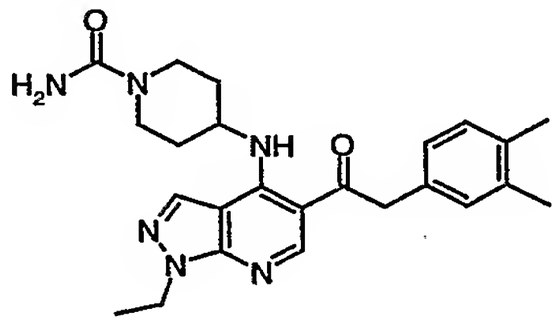
**<u>Example 1:</u> 4-{[1-(aminocarbonyl)-4-piperidinyl]amino} -N- [(2,4-dimethylphenyl)methyl] -1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide**



A solution of Intermediate 5 (0.066mmol) in DMF (1ml) was treated with EDC (0.066mmol), HOBT (0.066mmol) and DIPEA (0.151mmol) followed by 2,4-methyl benzylamine (0.066mmol). The reaction mixture was left to stand at 22°C for 16h. The DMF was evaporated and the residue was partitioned between DCM (5ml) and saturated aqueous $NaHCO_3$ (2ml). The organic layer was collected through a hydrophobic frit and evaporated. The residue was purified by mass directed autoprep. HPLC to give the title compound as a gum (7.9mg). LCMS showed $MH^+ = 450$; $T_{RET} = 2.8$min.

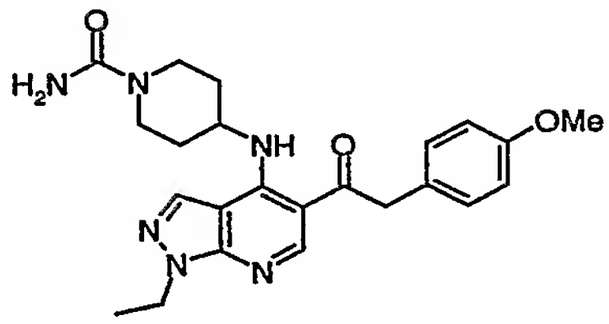
The following Example 2 was prepared from Intermediate 5 and 3,4-dimethylbenzylamine using this procedure:

**Example 2:4-{[1-(aminocarbonyl)-4-piperidinyl]amino}-N-[(3,4-dimethylphenyl)methyl]-1-ethyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide**



LCMS showed $MH^+ = 450$; $T_{RET} = 2.8$min.

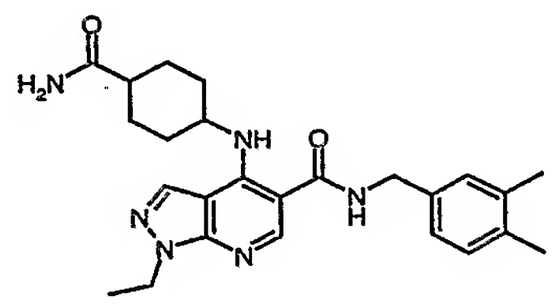
**Example 3: 4-{[1-(aminocarbonyl)-4-piperidinyl]amino}-1-ethyl-N-{[4-(methyloxy)phenyl]methyl}-1H-pyrazolo[3,4-b]pyridine-5-carboxamide**



A mixture of Intermediate 10 (27mg) and intermediate 12 (16mg) in MeCN (2ml) was treated with DIPEA (35µL). The reaction mixture was heated under reflux for 72h. The solvent was evaporated and the residue was partitioned between DCM (5ml) and saturated aqueous $NaHCO_3$ (2ml). The organic layer was collected through a hydrophobic frit and evaporated. The residue was purified by mass directed autoprep. HPLC to give Example 3 as a white solid (7.9mg). LCMS showed $MH^+ = 452$; $T_{RET} = 2.57$min.

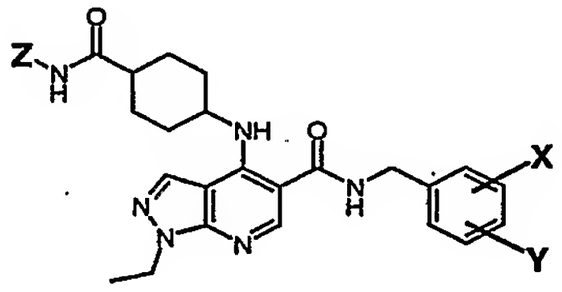
Examples 1 and 2 were also prepared using this procedure.

**Example 4: 4-{[4-(aminocarbonyl)cyclohexyl]amino}-*N*-[(3,4-dimethylphenyl)methyl]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide**



A solution of Intermediate 9 (0.08mmol) in MeCN (1ml) was treated with Intermediate 15 (0.088mmol) and DIPEA (0.2mmol). The reaction mixture was heated at reflux for 20h. The solvents were evaporated and the residue was partitioned between DCM (5ml) and water (2ml). The organic phase was collected through a hydrophobic frit and evaporated. The residue was purified by mass directed autoprep. HPLC to give Example 4 as a white solid (23mg). LCMS showed $MH^+ = 449$; $T_{RET} = 2.8$min.

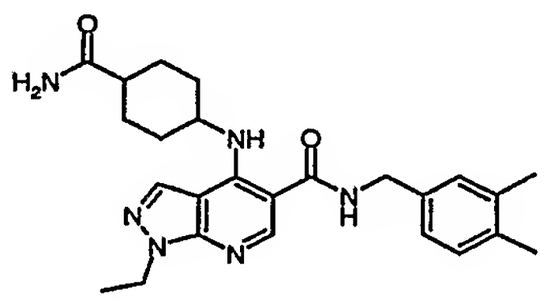
The following Examples 5-7 were prepared from Intermediates 8, 9, 15 and 16 using a similar procedure:



| Example Number | Input aryl chloride | Input cyclohexylamine | X,Y | Z | $MH^+$ Ion | LC-MS retention time |
|---|---|---|---|---|---|---|
| **5** | Intermediate 8 | Intermediate 15 | 2,4-$Me_2$ | H | 449 | 2.9 |
| **6** | Intermediate 8 | Intermediate 16 | 2,4-$Me_2$ | Me | 463 | 3.0 |
| **7** | Intermediate 9 | Intermediate 16 | 3,4-$Me_2$ | Me | 463 | 3.0 |

***Alternative Preparation of Example 4 and separation into cis and trans isomers (Examples 8 and 9)***

**4-{[4-(aminocarbonyl)cyclohexyl]amino}-*N*-[(3,4-dimethylphenyl)methyl]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide**



A solution of Intermediate 9 (75mg) in MeCN (5ml) was treated with Intermediate 15 (42mg) and DIPEA (200µL). The reaction mixture was heated at reflux for 16h. The solvents were evaporated and the residue was partitioned between DCM (10ml) and water

(5ml). The organic fraction was collected through a hydrophobic frit and evaporated. The residue was separated into its *cis* and *trans* isomers by purification on a 25cm Chiralpak AS column, eluting with 40% EtOH : 60% heptane mixture to give the isomers.

**Example 8: (cis)- 4-{[4-(aminocarbonyl)cyclohexyl]amino}-*N*-[(3,4-dimethylphenyl)methyl]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide**

First isomer to elute: isolated as a white solid (7.4mg). LCMS showed $MH^+ = 449$; $T_{RET}$ = 2.8min. Confirmation of the *cis*-relationship between the substituents on the cyclohexane ring was demonstrated by NMR (1D TOCSY experiments).

**Example 9: (trans)- 4-{[4-(aminocarbonyl)cyclohexyl]amino}-*N*-[(3,4-dimethylphenyl)methyl]-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide**

Second isomer to elute: isolated as a white solid (7.8mg). LCMS showed $MH^+ = 449$; $T_{RET}$ = 2.8min. Confirmation of the *trans*-relationship between the substituents of the cyclohexane ring was demonstrated by NMR (1D TOCSY experiments).

**Pharmaceutical Compositions**

**A Micronisation Example:**

- Purpose: To micronize a compound of formula (I), usually in an amount of approximately 600-1000 mg thereof, using a Jetpharma MC1 micronizer.
- The parent (unmicronised) and micronised materials are analyzed for particle size by laser diffraction and crystallinity by PXRD.

*Equipment and material*

| Equipment/material | Description and specification |
|---|---|
| Jetpharma MC1 Micronizer | Nitrogen supply: Air tank with 275psi rate tubing |
| Analytical balance | Sartorius Analytical |
| Top loader balance | Mettler PM400 |
| Digital Caliper | VWR Electronic caliper |
| Vibrational spatula | Auto-spat Dispenser |
| Materials to be micronised | A compound of formula (I) |

The Jetpharma MC1 Micronizer comprises a horizontal disc-shaped milling housing having: a tubular compound inlet (e.g. angled at ca. 30 degrees to the horizontal) for entry of a suspension of unmicronised compound of formula (I) or salt in a gasflow, a separate gas inlet for entry of gases, a gas outlet for exit of gases, and a collection vessel for collecting micronised material. The milling housing has two chambers: (a) an outer annular chamber in gaseous connection with the gas inlet, the chamber being for receiving pressurised gas (e.g. air or nitrogen), and (b) a disc-shaped inner milling chamber within and coaxial with the outer chamber for micronising the input compound / salt, the two chambers being separated by an annular wall. The annular wall (ring R) has a plurality of narrow-bored holes connecting the inner and outer chambers and circumferentially-spaced-apart around the annular wall. The holes opening into the inner chamber are directed at an angle (directed part-way between radially and tangentially), and in use act as nozzles directing pressurised gas at high velocity from the outer chamber into the inner chamber and in an inwardly-spiral path (vortex) around the inner chamber (cyclone). The compound inlet is in gaseous communication with the inner chamber via a nozzle directed tangentially to the inner chamber, within and near to the annular wall / ring R. Upper and lower broad-diameter exit vents in the central axis of the inner milling chamber connect to (a) (lower exit) the collection vessel which has no air outlet, and (b) (upper exit) the gas outlet which leads to a collection bag, filter and a gas exhaust. Inside and coaxial with the tubular compound inlet and longitudinally-movable within it is positioned a venturi inlet (V) for entry of gases. The compound inlet also has a bifurcation connecting to an upwardly-directed material inlet port for inputting material.

In use, the narrow head of the venturi inlet (V) is preferably positioned below and slightly forward of the material inlet port so that when the venturi delivers pressurised gas (e.g. air or nitrogen) the feed material is sucked from the material inlet port into the gasstream thorough the compound inlet and is accelerated into the inner milling chamber tangentially at a subsonic speed. Inside the milling chamber the material is further accelerated to a supersonic speed by the hole/nozzle system around the ring (R ) (annular wall) of the milling chamber. The nozzles are slightly angled so that the acceleration pattern of the material is in the form of an inwardly-directed vortex or cyclone. The material inside the milling chamber circulates rapidly and particle collisions occur during the process, causing larger particles to fracture into smaller ones. "Centrifugal" acceleration in the vortex causes the larger particles to remain at the periphery of the inner chamber while progressively smaller particles move closer to the center until they exit the milling chamber, generally through the lower exit, at low pressure and low velocity. The particles that exit the milling chamber are heavier than air and settle downward thorugh the lower exit into the collection vessel, while the exhaust gas rises (together with a minority of small particles of micronised material) and escapes into the atmosphere at low pressure and low velocity.

*Procedure:*

The micronizer is assembled. The venturi protrusion distance from input port is preferably adjusted to about 1.0 cm respectively (e.g. so that the narrow head of the venturi inlet is positioned below and slightly forward of the material inlet port) and is measured with a micro-caliper to make sure that it is inserted correctly. The ring (R ) and venturi (V) pressures are adjusted according to the values specified in the experimental design (refer to experimental section below) by adjusting the valves on the pressure gauges on the micronizer. The setup is checked for leakage by observing if there is any fluctuation in the reading of the pressure gauges.

Note that the venturi (V) pressure is kept at least 2 bars greater than the ring (R ) pressure to prevent regurgitation of material, e.g. outwardly from the material inlet port.

Balance performance is checked with calibration weights. Specified amount of the parent material (see section on experimental run) is weighed into a plastic weigh boat. The material is then fed into the micronizer using a vibrational spatula (e.g. V-shaped in cross-section) at a specified feed rate. The material feeding time and equipment pressures are monitored during the micronization process.

Upon completion of the micronising run, the nitrogen supply is shut off and the collection bag is tapped to allow particles to settle into the recovery / collection vessel at the bottom of the micronizer. The collection bag is removed and set aside. The micronised powder in the recovery vessel (collection vessel) and the cyclone (above the recovery vessel) are collected separately into different weighed+labelled collection vials. The weight of the micronised material is recorded. The micronizer is disassembled and residual PDE4 compound on the micronizer inner surface is rinsed with 70/30 isopropyl alcohol / water and collected into a flask. The micronizer is then thoroughly cleaned by rinsing and wiping with suitable solvent and dried before subsequent runs are performed.

*Preferred or Optional Experimental Parameters*
Parent (unmicronised) material (Procedure 1):
Balance(s) Used: Sartorius analytical

| Procedure no. | Material input amount (g) | Venturi Pressure (V) / ring (R ) Pressure (bar) | Intended feed-rate | Time needed to feed material (min+sec) | Actual feed-rate (g/min) |
|---|---|---|---|---|---|
| 1 | ca. 0.9 g | V= 8 to 10 bar<br>R= 5.5 to 6 bar | 180 to 200 mg/min | | procedure not carried out |

The above optional parameters can be varied using the skilled person's knowledge.

*Results and/or observations*
% yield = [(Material from vessel + Material from cyclone)/Material input amount] x100
In general, very approximately 50-75% yields are achievable using this method, including material from collection vessel and material from inside walls of cyclone.

Procedure 1 includes possible parameters and conditions and has not been carried out.

**B Dry powder inhalable compositions**

For pharmaceutical compositions suitable and/or adapted for inhaled administration, it is preferred that the pharmaceutical composition is a dry powder inhalable composition. Such a composition can comprise a powder base such as lactose or starch, the compound of formula (I) or salt thereof (preferably in particle-size-reduced form, e.g. in micronised form), and optionally a performance modifier such as L-leucine, mannitol, trehalose and/or magnesium stearate. Preferably, the dry powder inhalable composition comprises a dry powder blend of lactose and the compound of formula (I) or salt thereof. The lactose is preferably lactose hydrate e.g. lactose monohydrate and/or is preferably inhalation-grade and/or fine-grade lactose. Preferably, the particle size of the lactose is defined by 90% or more (by weight or by volume) of the lactose particles being less than 1000 microns (micrometres) (e.g. 10-1000 microns e.g. 30-1000 microns) in diameter, and/or 50% or more of the lactose particles being less than 500 microns (e.g. 10-500 microns) in diameter. More preferably, the particle size of the lactose is defined by 90% or more of the lactose particles being less than 300 microns (e.g. 10-300 microns e.g. 50-300 microns) in diameter, and/or 50% or more of the lactose particles being less than 100 microns in diameter. Optionally, the particle size of the lactose is defined by 90% or more of the lactose particles being less than 100-200 microns in diameter, and/or 50% or more of the lactose particles being less than 40-70 microns in diameter. Most importantly, it is preferable that about 3 to about 30% (e.g. about 10%) (by weight or by volume) of the particles are less than 50 microns or less than 20 microns in diameter. For

example, without limitation, a suitable inhalation-grade lactose is E9334 lactose (10% fines) (Borculo Domo Ingredients, Hanzeplein 25, 8017 JD Zwolle, Netherlands).

In the dry powder inhalable composition, preferably, the compound of formula (I) or salt thereof is present in about 0.1% to about 70% (e.g. about 1% to about 50%, e.g. about 5% to about 40%, e.g. about 20 to about 30%) by weight of the composition.

An illustrative non-limiting example of a dry powder inhalable composition follows:

**C Dry Powder Formulation Example - Dry powder Lactose Blend Preparation**

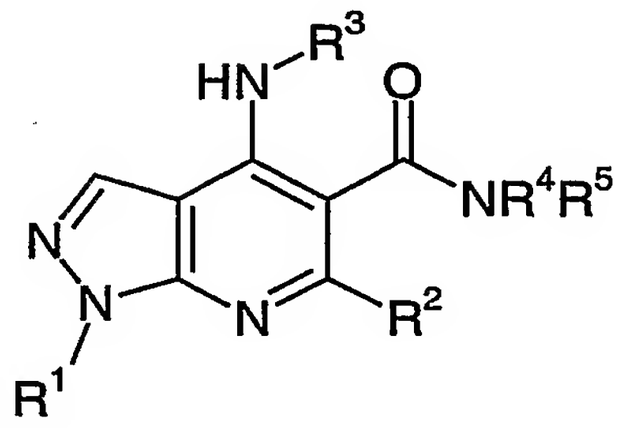
Using a size-reduced e.g. micronised form of the compound of formula (I) or salt thereof (e.g. as prepared in the Micronisation Example above), the dry powder blend is prepared by mixing the required amount of the compound/salt (e.g. 10 mg, 1% w/w) with inhalation-grade lactose containing 10% fines (e.g. 990 mg, 99% w/w) in a Teflon™ (polytetrafluoroethene) pot in a Mikro-dismembrator ball-mill (but without a ball bearing) at ¾ speed (ca. 2000-2500 rpm) for about 4 hours at each blend concentration. The Mikro-dismembrator (available from B. Braun Biotech International, Schwarzenberger Weg 73-79, D-34212 Melsungen, Germany; www.bbraunbiotech.com) comprises a base with an upwardly-projecting and sidewardly-vibratable arm to which is attached the Teflon $^{TM}$ pot. The vibration of the arm achieves blending.

Other blends: 10% w/w compound/salt (50 mg) + 90% w/w lactose (450 mg, inhalation-grade lactose containing 10% fines).

Serial dilution of the 1% w/w blend can achieve e.g. 0.1% and 0.3% w/w blends.

## Claims

1. a compound of formula (I) or a salt thereof (in particular, a pharmaceutically acceptable salt thereof):



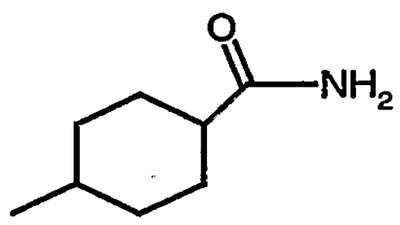
(I)

wherein:

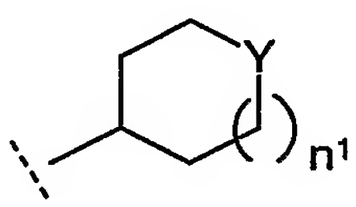
$R^1$ is $C_{1-4}$alkyl, $C_{1-3}$fluoroalkyl, or $-CH_2CH_2OH$;

$R^2$ is a hydrogen atom (H), methyl or $C_1$fluoroalkyl;

$R^3$ is a 4-(aminocarbonyl)cyclohexyl (i.e. 4-(aminocarbonyl)cyclohexan-1-yl) group of sub-formula (aa), or an N-aminocarbonyl-piperidinyl or -pyrrolidinyl group of sub-formula (bb);



(aa)



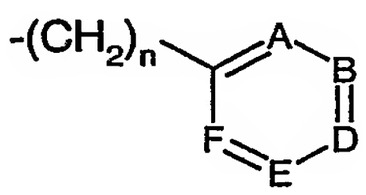
(bb)

wherein Y is $NHCONH_2$ and $n^1$ is 0 or 1;

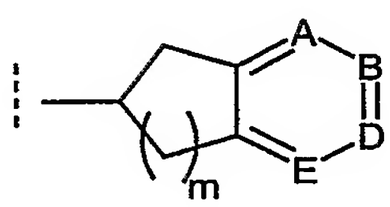
and wherein, the cyclohexyl group of sub-formula (aa) or the piperidinyl or pyrrrolidinyl groups of sub-formula (bb) may be further optionally substituted with one or two substituents independently selected from $C_{1-2}$alkyl; $C_{1-2}$fluoroalkyl; $CH_2OH$; $-C(O)OR^{23}$ wherein $R^{23}$ is H or $C_{1-2}$alkyl; $-C(O)NHR^{23}$; or fluoro; on any ring carbon; as well as, on the C2, C3, C5 and C6 of the cyclohexyl group of (aa), the C2 or C6 of the piperidinyl ring or the C5 of the pyrrolidinyl ring of (bb), a substituent selected from OH; $C_{1-2}$alkoxy; $C_{1-2}$fluoroalkoxy; OH, alkoxy;

$R^4$ is a hydrogen atom (H); $C_{1-6}$alkyl; $C_{1-3}$fluoroalkyl; or $C_{2-6}$alkyl substituted by one substituent $R^{11}$;

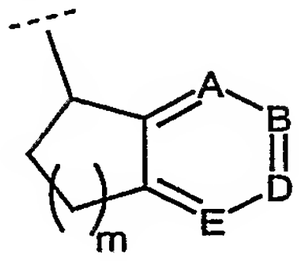
$R^5$ is a group of the sub-formula (x), (y), (y1) or (z):



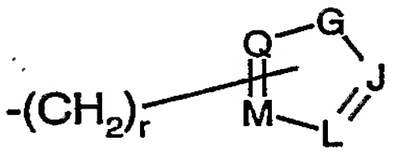
(x)



(y)



(y1)



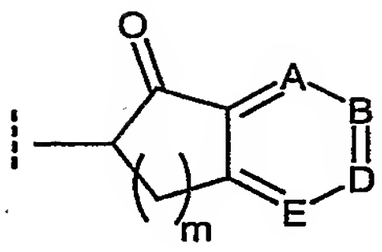
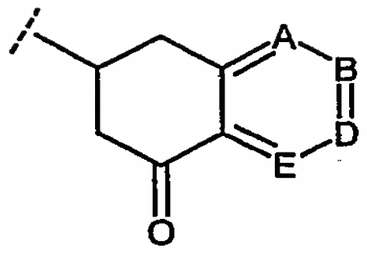
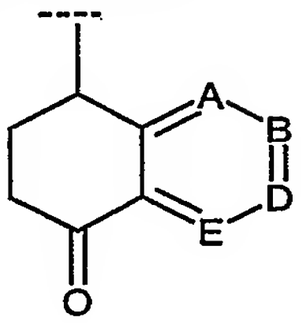
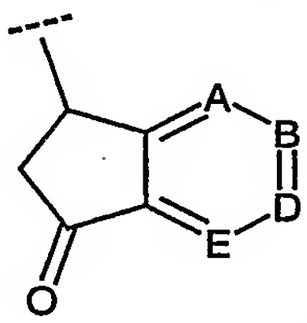
(z)

wherein in sub-formula (x), n = 0, 1 or 2; in sub-formula (y) and (y1), m = 1 or 2; and in sub-formula (z), r = 0, 1 or 2;

wherein in sub-formula (x) and (y) and (y1), none, one or two of A, B, D, E and F are independently nitrogen or nitrogen-oxide ($N^+$-$O^-$) provided that no more than one of A, B, D, E and F is nitrogen-oxide; and the remaining of A, B, D, E and F are independently CH or $CR^6$;

wherein, each $R^6$, independently of any other $R^6$ present, is: a halogen atom; $C_{1-6}$alkyl (e.g. $C_{1-4}$alkyl or $C_{1-2}$alkyl); $C_{1-4}$fluoroalkyl (e.g. $C_{1-2}$fluoroalkyl); $C_{1-4}$alkoxy (e.g. $C_{1-2}$alkoxy); $C_{1-2}$fluoroalkoxy; $C_{3-6}$cycloalkyloxy; $-C(O)R^{16a}$; $-C(O)OR^{30}$; $-S(O)_2-R^{16a}$ (e.g. $C_{1-2}$alkylsulphonyl, that is $C_{1-2}$alkyl-$SO_2$-); $R^{16a}$-$S(O)_2$-$NR^{15a}$- (e.g. $C_{1-2}$alkyl-$SO_2$-NH-); $R^7R^8N$-$S(O)_2$-; $C_{1-2}$alkyl-C(O)-$R^{15a}N$-$S(O)_2$-; $C_{1-4}$alkyl-S(O)-, Ph-S(O)-, $R^7R^8N$-CO-; $-NR^{15}$-$C(O)R^{16}$; $R^7R^8N$; OH; $C_{1-4}$alkoxymethyl; $C_{1-4}$alkoxyethyl; $C_{1-2}$alkyl-$S(O)_2$-$CH_2$-; $R^7R^8N$-$S(O)_2$-$CH_2$-; $C_{1-2}$alkyl-$S(O)_2$-$NR^{15a}$-$CH_2$-; $-CH_2$-OH; $-CH_2CH_2$-OH; $-CH_2-NR^7R^8$; $-CH_2-CH_2-NR^7R^8$; $-CH_2-C(O)OR^{30}$; $-CH_2-C(O)-NR^7R^8$; $-CH_2-NR^{15a}$-C(O)-$C_{1-3}$alkyl; $-(CH_2)_n{}^{14}$-$Het^1$ where $n^{14}$ is 0 or 1; cyano (CN); $Ar^{5b}$; or phenyl, pyridinyl or pyrimidinyl wherein the phenyl, pyridinyl or pyrimidinyl independently are optionally substituted by one or two of fluoro, chloro, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy; or where two adjacent $R^6$ taken together are –O–($CMe_2$)–O– or –O–$(CH_2)_n{}^{14}$–O– where $n^{14}$ is 1 or 2;

wherein sub-formula (y) and (y1), independently, are optionally substituted by oxo (=O) at a ring carbon adjacent the 6-membered aromatic ring (for example, sub-formula (y) can

optionally be  or  , or sub-formula (y1) can optionally be  or  );

wherein in sub-formula (z), G is O or S or $NR^9$ wherein $R^9$ is a hydrogen atom (H), $C_{1-4}$alkyl or $C_{1-4}$fluoroalkyl; none, one, two or three of J, L, M and Q are nitrogen; and the remaining of J, L, M and Q are independently CH or $CR^6$ where $R^6$, independently of any other $R^6$ present, is as defined herein;

and wherein:

$R^7$ and $R^8$ are independently a hydrogen atom (H); $C_{1-4}$alkyl (e.g. $C_{1-2}$alkyl such as methyl); $C_{3-6}$cycloalkyl; or phenyl optionally substituted by one or two substituents independently being: fluoro, chloro, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy;

or $R^7$ and $R^8$ together are $-(CH_2)_n{}^6-$ or $-C(O)-(CH_2)_n{}^7-$ or $-C(O)-(CH_2)_n{}^{10}-C(O)-$ or $-(CH_2)_n{}^8-X^7-(CH_2)_n{}^9-$ or $-C(O)-X^7-(CH_2)_n{}^{10}-$ in which: $n^6$ is 3, 4, 5 or 6 (suitably $n^6$ is 4 or 5), $n^7$ is 2, 3, 4, or 5 (suitably $n^7$ is 3 or 4), $n^8$ and $n^9$ and $n^{10}$ independently are 2 or 3 (suitably independently 2), and $X^7$ is O or $NR^{14}$;

$R^{7a}$ is a hydrogen atom (H) or $C_{1-4}$alkyl (suitably H or $C_{1-2}$alkyl, more suitably H or methyl);

$R^{8a}$ is a hydrogen atom (H) or methyl (suitably H);

$R^{14}$, independent of other $R^{14}$, is a hydrogen atom (H); $C_{1-4}$alkyl (e.g. $C_{1-2}$alkyl); $C_{1-2}$fluoroalkyl (e.g. $CF_3$); cyclopropyl; $-C(O)-C_{1-4}$alkyl (e.g. -C(O)Me); $-C(O)NR^{7a}R^{8a}$ (e.g. $-C(O)NH_2$); or $-S(O)_2-C_{1-4}$alkyl (e.g. $-S(O)_2$Me) (preferably, $R^{14}$, $R^{17}$ and/or $R^{17a}$ independently is/are: H; $C_{1-2}$alkyl; or -C(O)Me);

$R^{15}$, independent of other $R^{15}$, is a hydrogen atom (H); $C_{1-4}$alkyl (e.g. $^t$Bu or $C_{1-2}$alkyl e.g. methyl); $C_{3-6}$cycloalkyl; or phenyl optionally substituted by one or two of: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy;

$R^{15a}$ , independent of other $R^{15a}$, is a hydrogen atom (H) or $C_{1-4}$alkyl (e.g. H, $^tBu$ or $C_{1-2}$alkyl such as methyl; preferably $R^{15a}$ is H or $C_{1-2}$alkyl, more preferably H);

$R^{16a}$ is:
$C_{1-6}$alkyl (e.g. $C_{1-4}$alkyl or $C_{1-2}$alkyl);
$C_{3-6}$cycloalkyl (e.g. $C_{5-6}$cycloalkyl) optionally substituted by one oxo (=O), OH or $C_{1-2}$alkyl substituent (e.g. optionally substituted at the 3- or 4-position of a $C_{5-6}$cycloalkyl ring; and/or preferably unsubstituted $C_{3-6}$cycloalkyl);
$C_{3-6}$cycloalkyl-$CH_2$- (e.g. $C_{5-6}$cycloalkyl-$CH_2$-);
pyridinyl (e.g. pyridin-2-yl) optionally substituted on a ring carbon atom by one of: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy;
$Ar^{5c}$;
phenyl optionally substituted by one or two substituents independently being: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy;
benzyl optionally substituted on its ring by one or two substituents independently being: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, $C_{1-2}$alkoxy or $C_1$fluoroalkoxy; or
a 4-, 5-, 6- or 7-membered saturated heterocyclic ring connected at a ring-carbon and containing one or two ring-hetero-atoms independently selected from O, S, and N; wherein any ring-nitrogens which are present are present as $NR^{27}$ where $R^{27}$ is H, $C_{1-2}$alkyl or -C(O)Me; and wherein the ring is optionally substituted at carbon by one $C_{1-2}$alkyl or oxo (=O) substituent, provided that any oxo (=O) substituent is substituted at a ring-carbon atom bonded to a ring-nitrogen;

$R^{30}$, independent of other $R^{30}$, is a hydrogen atom (H), $C_{1-4}$alkyl or $C_{3-6}$cycloalkyl;

$Ar^{5b}$ and $Ar^{5c}$ independently is/are a 5-membered aromatic heterocyclic ring containing one O, S or $NR^{15a}$ in the 5-membered ring, wherein the 5-membered ring can optionally additionally contain one or two N atoms, and wherein the heterocyclic ring is optionally substituted on a ring carbon atom by one of: a halogen atom, $C_{1-2}$alkyl, $C_1$fluoroalkyl, -$CH_2$OH, -$CH_2$-O$C_{1-2}$alkyl, OH (including the keto tautomer thereof) or –$CH_2$-$NR^{28}R^{29}$ wherein $R^{28}$ and $R^{29}$ independently are H or methyl; and

$Het^1$ is a 4-, 5-, 6- or 7-membered saturated heterocyclic ring connected at a ring-carbon and containing one or two ring-hetero-atoms independently selected from O, S, and N; wherein any ring-nitrogens which are present are present as $NR^{31}$ where $R^{31}$ is H, $C_{1-2}$alkyl or -C(O)Me; and wherein the ring is optionally substituted at carbon by one $C_{1-2}$alkyl or oxo (=O) substituent, provided that any oxo (=O) substituent is substituted at a ring-carbon atom bonded to a ring-nitrogen.